Shedding Light on the Histamine H3 Receptor

Mocking, T.A.M.

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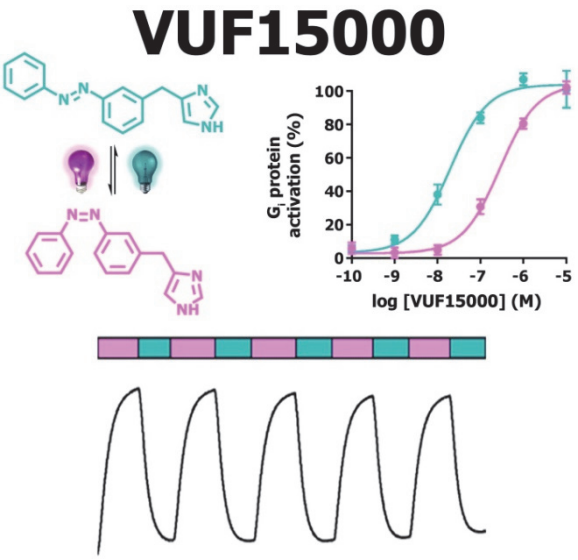
Chapter 7

A Photoswitchable Agonist for the Histamine H_3 Receptor, a prototypic family A G protein-coupled receptor



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Authored by: Tamara A.M. Mocking[+], Niels J. Hauwert[+], Daniel Da Costa Pereira, Ken Lion, Yara Huppelschoten, Henry F. Vischer, Iwan J.P. De Esch, Maikel Wijtmans, Rob Leurs

[+] authors contributed equally

Abstract

Spatiotemporal control over biochemical signaling processes involving G protein-coupled receptors (GPCRs) is highly desired for dissecting their complex intracellular signaling. We developed a set of sixteen photoswitchable ligands for the human histamine H_3 receptor (hH_3R). Upon illumination key compound **65** decreases its affinity for the hH_3R 8.5-fold and its potency in hH_3R-mediated G_i protein activation over 20-fold, with the *trans* and *cis* isomer both acting as full agonist. Furthermore, in real-time two-electrode voltage clamp experiments in *Xenopus* oocytes, **65** shows rapid light-induced modulation of hH_3R activity. Ligand **65** shows good binding selectivity amongst the histamine receptor subfamily and has good photolytic stability. In all, **65** (VUF15000) is the first photoswitchable GPCR agonist confirmed to be modulated through its affinity and potency upon photoswitching while maintaining its intrinsic activity, rendering it a new chemical biology tool for spatiotemporal control of GPCR activation.

Introduction:

In recent years, photopharmacology has been gaining momentum as a strategy to optically control biochemical processes [1,2]. The use of light as an external trigger to change ligand shape and consequently its pharmacological properties allows the probing of biological systems with great spatiotemporal resolution [3]. The azobenzene moiety is often used in photoswitchable ligands [1] due to its limited size, high photostability and tunability of the peak absorption wavelength λ_{max} [4]. Its thermodynamically stable *trans* isomer typically involves a flat elongated structure, whereas its photo-induced *cis* configuration has a bent geometry with considerably shorter end-to-end distance [5]. Whereas photopharmacology is well established in the field of enzyme and ion channel modulation, it is an upcoming technology for G protein-coupled receptors (GPCRs) [1]. GPCRs constitute one of the largest families of transmembrane proteins, their dysfunction is associated with a plethora of diseases and consequently GPCRs are one of the most successful classes of drug targets [6,7]. Recently, various GPCRs have been successfully targeted using photopharmacology approaches, including μ-opioid[8], CXCR3[9], CB1[10], H_3R[11], mGlu5[12] and GLP1[13]. Yet, almost all these examples include at least one but more frequently two antagonistic/partial agonist isomeric forms. In contrast, freely diffusible affinity and potency photoswitches in which both isomers act as full agonists are scarce[1], even though such compounds would be very useful for photopharmacology approaches and complementary to agonist-to-antagonist switches.

The histamine H_3R receptor is an intensively studied GPCR that is known to play an important role in sleep disorders and cognition-related diseases such as Alzheimer's and Parkinson's disease. The first H_3R antagonist pitolisant (Wakix®) has been approved by the European Medicines agency for the treatment of narcolepsy [14–19]. Recently, we published a toolbox of photoswitchable antagonists that competitively inhibit histamine-induced H_3R activity (Chapter 5) [11]. In the current work, we aimed to develop high-potency H_3R photoswitchable agonists that can simplify spatiotemporal studies of the signalling network of the H_3R. We disclose unique photoswitchable H_3R agonists that can be optically converted into different isomers that differ in their affinity and potency.

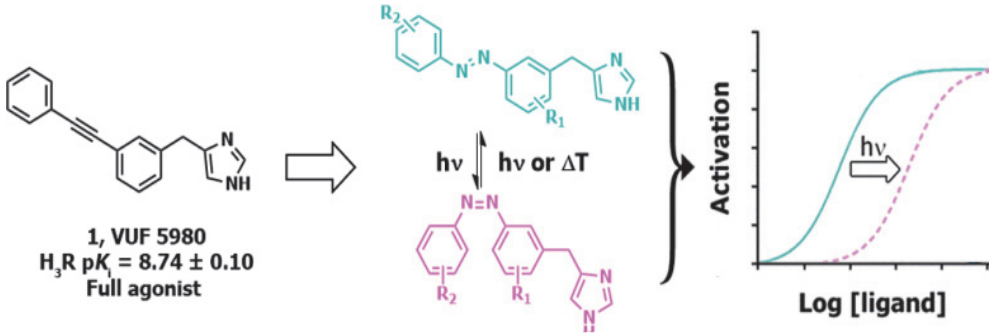
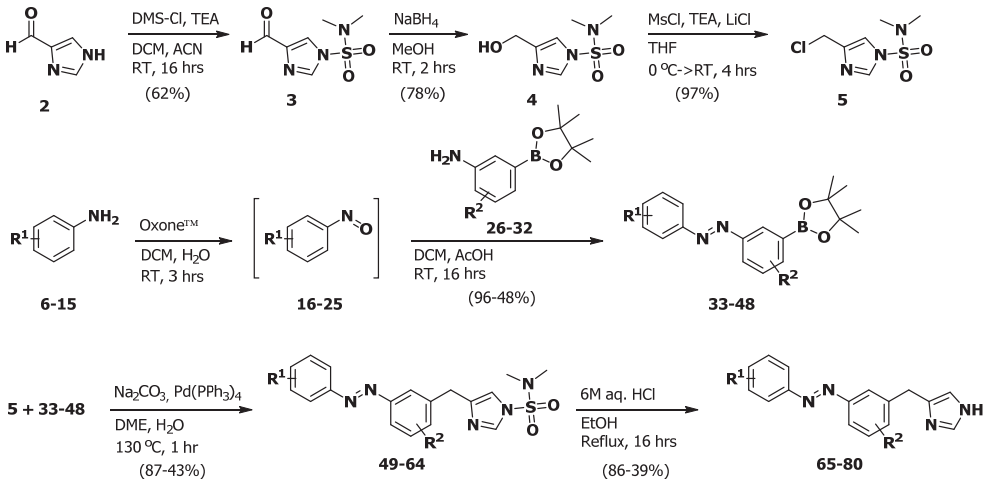


Figure 1. General design and concept of photoswitchable H_3R full agonists.



Scheme 1. General synthetic scheme for photoswitchable H_3R agonists. See supporting information for detailed experimental procedures.

Results and discussion

The scaffold design was inspired by hH_3R full agonist VUF5980 previously published by our lab (Figure 1) [20]. To date, virtually every published hH_3R full agonist contains a 4-substituted-imidazole moiety combined with a basic or neutral side chain, as is the case for VUF5980. We left the imidazole portion of the molecule unchanged, and considered the diphenylacetylene moiety to be an attractive candidate for an "azologization" strategy[4]. Introducing the azobenzene at this position furthermore allows for great flexibility in the diversification of the scaffold. Based on the steep structure-activity relationship observed with VUF5980[20] it was postulated that small changes would have significant impact on the affinity and potency for hH_3R. Therefore, primarily the azobenzene was decorated with small substituents (i.e. methyl and fluorine groups) on both phenyl rings.

To synthesize the ligands, imidazole-4-carbaldehyde **2** was protected using *N,N*-dimethylsulfamoylchloride (DMS-Cl, Scheme 1) to afford **3**, which was reduced to **4**. Alcohol **4** was converted to chloride **5** using an *in situ* mesylation. A diverse set of anilines **6-15** was oxidized to the corresponding nitrosobenzenes **16-25** using Oxone™. After work-up, they were directly used in a Mills reaction with 3-amino-phenylboronic acid pinacol esters **26-32** to yield azobenzene-pinacol esters **33-48.** Cross coupling with chloride **5** yielded **49-64** in generally good yields. Acidic deprotection yielded final compounds **65-80** which were used for biological evaluation.

Compounds **65-80** all have λ_{max} values for the π-π* transition of the *trans* isomer between 313 and 330 nm (Table 1). The observed limited variation is due to the absence of strong electron-donating or -withdrawing substituents. Similarly, λ_{max} values for the n-π* transition

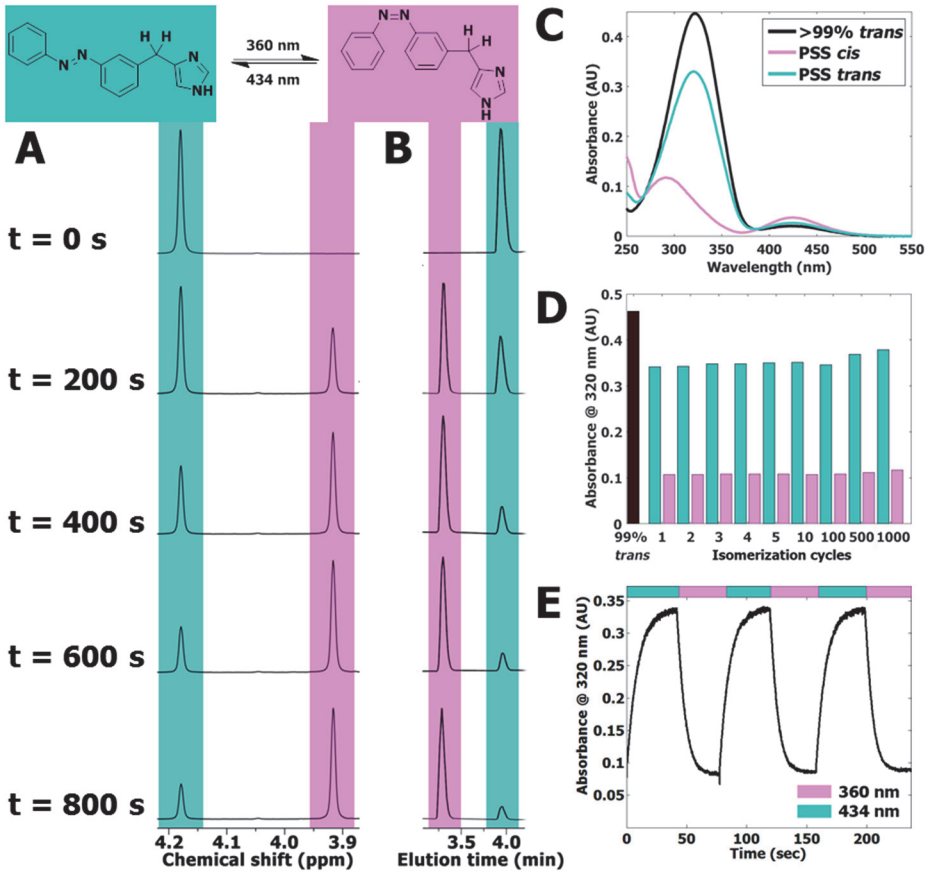


Figure 2. (A) Representative part of ^{1}H NMR spectra of 10 mM 65 in DMSO-d6 illuminated at 360 ± 20 nm displayed at various time points (seconds). The presented peak belongs to the hydrogen atoms explicitly drawn in the structure shown above the spectrum. Full spectra are available in Figure S4. (B) Representative part of LC–MS chromatograms belonging to the illuminated NMR sample in Figure 2A. Full chromatograms are available in Figure S5. (C) UV–Vis spectra of 25 µM of 65 in 50 mM Tris–HCl pH 7.4 buffer + 1% DMSO-d6. PSS cis represents a sample which has been illuminated for 300 s using 360 ± 20 nm light. PSS trans represents subsequent illumination for 300 s using 434 ± 9 nm. (D) Repeated isomerization of 25 µM of 65 in 50 mM Tris–HCl pH 7.4 buffer + 1% DMSO-d6 analyzed at 320 nm. PSS cis was obtained by using illuminations for 40 s at 360 ± 20 nm. PSS trans was obtained by using illuminations for 40 s at 434 ± 9 nm. (E) Absorbance at 320 nm of 25 µM of 65 in 50 mM Tris–HCl pH 7.4 buffer + 1% DMSO-d6. UV–Vis spectra were obtained with 1 s intervals under alternating illumination with 360 ± 20 nm and 434 ± 9 nm perpendicular to the light source of the UV–Vis spectrometer.

of the *cis* isomer differed marginally, ranging between 417 and 430 nm. Upon continuous illumination with 360 ± 20 nm, the values for the photostationary states (PSS) of **65-80** ranged between 92.3 and 97.5% *cis*, except for **69** which has 82.6% PSS. Compounds **65-80** all showed slow thermal relaxation at room temperature (20 °C, Table 1). The observed thermal relaxation half-lives were impractically long for direct quantification, therefore extrapolations of high temperature thermal relaxation were used to quantify half-lives at

20 °C (Table 1, Figure S1). Compound **69** shows the fastest thermal relaxation in 50 mM Tris-HCl pH 7.4 buffer, having a half-life of 26.6 days, while **72** shows the slowest relaxation with a half-life of 147 days.

Based on its favorable pharmacological profile (*vide infra*) and synthetic tractability, compound **65** was subjected to in-depth photochemical characterization using ^{1}H-NMR and LC-MS analysis during illumination with 360 ± 20 nm. The well-resolved signal of the benzylic CH_2 group provided a clear handle for quantification in ^{1}H-NMR analysis (Figure 2A). An overestimation of isomerization percentage is observed in LCMS analysis at 254 nm compared to ^{1}H-NMR analysis (Figure 2B, Table S1), which can be explained by the differences in extinction coefficients for *trans* and *cis* isomer at 254 nm (Figure 2C, S2, S3). Compound **65** shows excellent resistance to photobleaching during more than 1000 isomerization cycles (Figure 2D). The dynamic isomerization was studied using UV-Vis spectroscopy under alternating illumination (Figure 2E). At 25 µM of **65** in 50 mM Tris-HCl pH 7.4 buffer + 1% DMSO-d_6, a half-life of 4.2 ± 0.16 s for 360 ± 20 nm and 5.7 ± 0.19 s for 434 ± 9 nm was observed.

The long thermal relaxation half-lives allowed for detailed pharmacological evaluation using hH_3R competition binding as well as functional experiments. For this, the compound solutions were either illuminated using 360 ± 20 nm to reach a PSS *cis* or kept in the dark to ensure >99% *trans* isomer. The affinity of both isomers for the hH_3R was assessed in competition binding with [^{3}H]-N$^\alpha$-methylhistamine (NAMH). All compounds display hH_3R binding affinity, which decreased upon illumination reaching up to a 21-fold affinity difference in the case of **76**. In terms of absolute affinity, **65** displays the highest affinities for the hH_3R with a pK_i value of 8.42 ± 0.04 for its *trans* isomer and a pK_i of 7.49 ± 0.05 for its *cis* isomer, resulting in an 8.5 fold shift upon illumination (Figure 3A, Table 1). Fluorine-substituted analogues **67** and **80** performed similarly to **65** in competition binding displaying only a marginally lower affinity (Table 1). Notably, *para*-methyl substitution on the R^1 position (**78**) decreased the binding affinity and abrogated the photoisomerization-induced affinity shift compared to **65** (Table 1). Reduction of the size of the *para*-substituents to either chlorine (**71**) or fluorine (**68**) moieties gradually rescues hH_3R affinity and reestablishes the shift in affinity to 6- and 15-fold, respectively. Methylation at either the *ortho* (**76**) or *meta* (**77**) position of R^1 still resulted in decent binding affinities and high (21-fold) to good (8.5-fold) affinity shifts upon illumination. Addition of substituents at the R^2 position resulted in a clear affinity cliff, with fluorine substitutions (**79** and **80**) still being allowed, but the addition of a methyl substituent (**72**-**75**) highly decreases the binding affinity of the *cis* isomer. Moreover, for the *trans* isomers, 4-Me (**73**) and 6-Me (**75**) substitution is still tolerated yet showing a log-unit decrease in hH_3R affinity compared to **65**, while 2-Me (**72**) and 5-Me (**74**) groups highly reduced hH_3R affinity and consequently reduced or even abolished (**72**) the affinity shift (Table 1).

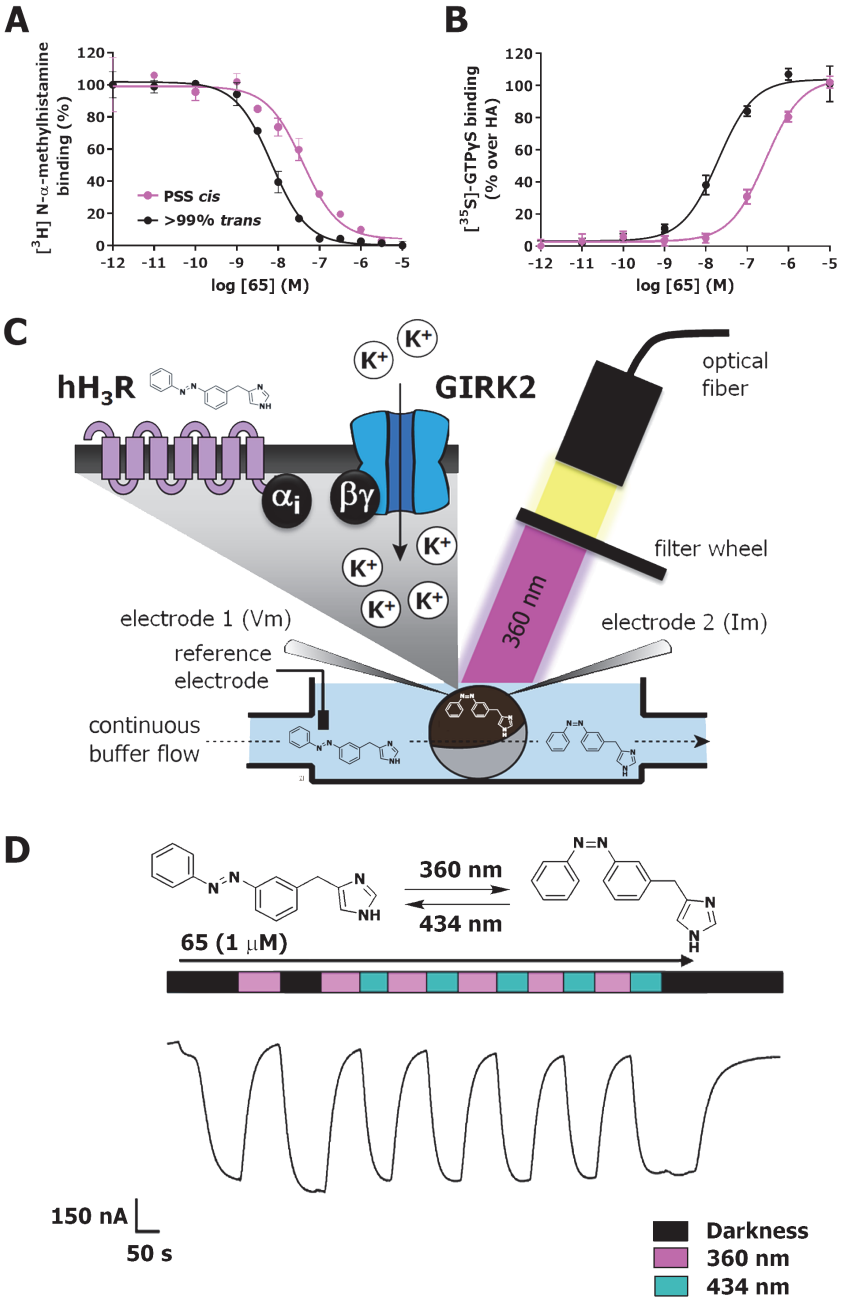


Figure 3. Representative curves of 65 (A) in competition binding with [^{3}H]-NAMH or (B) in G_i protein activation as measured by [^{35}S]-GTPγS accumulation on HEK293T cell homogenates transiently expressing hH_3R. Black lines refer to a sample containing >99% trans, while magenta lines refer to a sample illuminated to PSS with 360 ± 20 nm prior to the assay. (C) Schematic drawing of the TEVC setup used for dynamic hH_3R and GIRK current activation in Xenopus laevis oocytes expressing hH_3R and GIRK. (D) Representative part of a GIRK-mediated current trace during continuous perfusion with 1 μM 65 under illumination of the oocyte with alternating 360 ± 20 and 434 ± 9 nm wavelength as measured by TEVC. Error bars shown are mean ± SD.

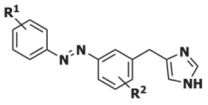
Based on the observed affinities and photo-induced affinity shifts, the efficacy in stimulating hH_3R-mediated $G\alpha_i$ protein activation was evaluated for ligands **65** and **76** in a [^{35}S]-GTPγS binding assay. The highest affinity ligand **65** (pK_i *trans* = 8.42 ± 0.04) also displays the highest potency (pEC_{50} *trans* = 7.60 ± 0.13) to induce $G\alpha_i$ activation, which upon photoisomerization decreased 20-fold (pEC_{50} at PSS *cis*: 6.30 ± 0.13) with both isomers being full agonists and having intrinsic activities of α = 1.0 ± 0.03, compared to histamine (Figure 3B). Since the observed shift in hH_3R affinity was 8.5-fold, the larger (20-fold) shift in functional potency indicates that for **65** the efficacy (propensity to activate a GPCR[21]) is also affected upon *trans-cis* isomerization. Interestingly, a large photo-induced decrease in potency of 23-fold was also obtained for **76** (pEC_{50} *trans*: 6.78 ± 0.11, PSS *cis*: 5.41 ± 0.11, α = 1.00 ± 0.0). This shift in potency of **76** is completely explained by the observed change of its affinity (*vide infra*).

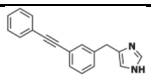
Compound **65** (VUF15000) was selected as tool compound for further analysis, as it has good synthetic tractability and its superior potency is a clear advantage for pharmacological studies. As the imidazole-based pharmacophore/scaffold used in the design of these photoswitchable ligands is prone to interact with other histamine receptor subtypes[22], **65** was tested for its subtype selectivity. Binding of **65** was more than 300-fold selective for hH_3R over hH_1R and hH_2R (Table S2), while a 30-fold selectivity was observed over its closest homologue hH_4R (Table S2). Interestingly, **65** displays high nM (*trans*) to low µM (PSS *cis*) binding affinities for both mouse and rat H_3R with a 4-fold and 8-fold shift in binding affinity upon photoisomerization, respectively (Table S2).

Real-time photomodulation of hH_3R activity by **65** was measured using two-electrode voltage clamp (TEVC) on *Xenopus laevis* oocytes expressing both hH_3R and G protein-coupled inwardly-rectifying potassium (GIRK)-channels (Figure 3C). In this expression system, histamine application results in hH_3R-mediated GIRK activation, which is insensitive to optical modulation[11]. As expected based on our data with the [^{35}S]-GTPγS binding assay, *trans*-**65** elicits an agonistic response in this system, which could be reduced by switching to the less active *cis* isomer upon illumination with 360 ± 20 nm. Retrieval of the agonistic response could be provoked by either actively switching the *cis*- back into its *trans* isomer by illuminating with 434 ± 9 nm or by stopping illumination, due to continuous perfusion of the *trans* isomer (Figure 3D). Dynamic photoswitching of **65** could be performed repeatedly, illustrating that the use of two specific wavelengths allows optical control of the hH_3R activation mediated by **65**. Furthermore, photoswitchable agonist **65** shows rapid hH_3R activation and deactivation kinetics, aiding in its use in *in vivo* experimentation.

In summary, we have synthesized and characterized 16 photoswitchable hH_3R agonists that change their affinity and potency upon illumination, indicating a successful azologization strategy. All possess long thermal relaxation half-lives at room temperature making them useful for a variety of pharmacological studies. Compound **65** (VUF15000) was selected as key compound on the basis of synthetic tractability and highest absolute

Table 1. Structure-affinity relationship of photoswitchable azobenzene-derived H_3R agonists.



Compound number	R^1	R^2	pK_i *trans* ± SEM	pK_i at PSS *cis*[a] ± SEM	pK_i shift	λ_{max} *trans* (nm) [b]	λ_{max} *cis* (nm) [b]	$t_{1/2}$ (days)[c]	PSS ± SEM[d]
1			8.74 ± 0.10[a]		-	-		-	-
65	H	H	8.42 ± 0.04	7.49 ± 0.05	-0.93 ± 0.06	320	427	106	96.1 ± 1.9
66	2-F	H	8.28 ± 0.08	7.09 ± 0.03	-1.19 ± 0.04	323	425	128	95.7 ± 0.27
67	3-F	H	8.35 ± 0.09	7.42 ± 0.05	-0.93 ± 0.04	320	425	101	94.1 ± 1.3
68	4-F	H	7.69 ± 0.08	6.51 ± 0.08	-1.18 ± 0.09	322	426	95.9	95.9 ± 1.6
69	2,6-F	H	8.00 ± 0.02	7.26 ± 0.09	-0.74 ± 0.10	313	417	26.6	82.6 ± 1.9
70	2-Cl	H	7.86 ± 0.03	6.85 ± 0.04	-1.02 ± 0.03	324	420	96.1	95.3 ± 0.22
71	4-Cl	H	6.76 ± 0.07	5.98 ± 0.07	-0.78 ± 0.10	326	428	29.7	97.5 ± 0.48
72	H	2-Me	5.57 ± 0.09	5.45 ± 0.03	-0.12 ± 0.10	323	428	147	92.3 ± 4.9
73	H	4-Me	6.90 ± 0.06	5.77 ± 0.13	-1.13 ± 0.08	327	430	42.9	96.3 ± 1.1
74	H	5-Me	5.75 ± 0.03	5.13 ± 0.17	-0.62 ± 0.19	322	427	122	94.5 ± 1.4
75	H	6-Me	7.15 ± 0.03	5.94 ± 0.06	-1.21 ± 0.04	324	426	125	95.8 ± 0.92
76	2-Me	H	7.72 ± 0.03	6.40 ± 0.04	-1.32 ± 0.05	326	426	35.6	96.5 ± 1.6
77	3-Me	H	7.39 ± 0.08	6.46 ± 0.06	-0.94 ± 0.09	323	428	77.0	95.4 ± 0.39
78	4-Me	H	5.72 ± 0.14	5.71 ± 0.06	-0.01 ± 0.16	330	429	34.1	94.0 ± 4.4
79	H	4-F	7.81 ± 0.07	6.54 ± 0.06	-1.27 ± 0.02	324	425	91.7	96.6 ± 0.51
80	H	6-F	8.39 ± 0.06	7.36 ± 0.03	-1.03 ± 0.03	322	427	84.6	94.6 ± 1.3

[a] Adapted from wijtmans et al. [20]. [b] Determined at 25 µM in 50 mM Tris-HCl pH 7.4 buffer + 1% DMSO-d_6. [c] Thermal relaxation half-life times as determined according to the method of Priimagi et al.[26] in 50 mM Tris-HCl pH 7.4 buffer + 1% DMSO-d_6 extrapolating to 20 °C. Arrhenius plots are available in Figure S1. [d] Photostationary state area percentages after illumination with 360 ± 20 nm at 1 mM in DMSO-d_6 and as determined by LC–MS analysis at 254 nm. All pharmacology experiments were at least performed as triplicate.

hH_3R affinity. Moreover, upon illumination **65** displays a high potency and 20-fold potency shift, while maintaining full intrinsic activity in $G\alpha_i$ protein activation, making it especially attractive as a tool compound. With a 20-fold shift in potency, **65** is one of the best photoswitchable GPCR agonists reported so far. Electrophysiology experiments showed the dynamic optical modulation of hH_3R activation induced by **65** in real time, setting the stage for further unraveling of the downstream signaling of hH_3R with great spatiotemporal precision. Recently, a photopharmacology approach with freely diffusible GPCR ligands have, for the first time, been used successfully in vivo to modulate tadpole and zebrafish behavior[12,23] and to elucidate the role of the metabotropic glutamate receptor 4 in the nervous system using a mouse model of chronic pain[24]. In view of the widespread distribution of the H_3R in the central and peripheral nervous system, photopharmacology approaches with tools such as **65** offer new means (complimentary to optogenetic approaches[25]) to investigate the spatial and temporal details of H_3R modulation of important processes, for example, arousal, cognition and neuropathic pain[14,16–19].

Materials and methods

Synthesis and characterization of compounds

All starting materials were obtained from commercial suppliers (primarily being Sigma-Aldrich, Fluorochem and Combi- Blocks) and used without purification. Anhydrous DCM, ACN and THF were obtained by passing through an activated alumina column prior to use. Anhydrous MeOH was purchased from Acros Organics (Geel, Belgium) and used without prior purification. All reactions were carried out under a nitrogen atmosphere unless mentioned otherwise. TLC analyses were performed using Merck F254 aluminum-backed silica plates and visualized with 254 nm UV light or a potassium permanganate stain. Flash column chromatography was executed using Silicycle Siliaflash F60 silica gel or by means of a Biotage Isolera equipment using Biotage SNAP columns. Microwave chemistry was performed using a Biotage Initiator+. All HRMS spectra were recorded on a Bruker micrOTOF mass spectrometer using ESI in positive-ion mode. All NMR spectra were recorded on either a Bruker Avance 250, Bruker Avance 300, Bruker Avance 400, Bruker Avance 500, or Bruker Avance 600 spectrometer. The peak multiplicities are defined as follows: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; p, pentet; dd, doublet of doublets; dt, doublet of triplets; td, triplet of doublets; bs, broad singlet; m, multiplet; app, apparent. The spectra were referenced to the internal solvent peak as follows:$CDCl_3$ (^1H = 7.26 ppm), ^{13}C = 77.16 ppm), DMSO-d_6 (^1H = 2.50 ppm, ^{13}C = 39.52 ppm)[27] or externally referenced to fluorobenzene in $CDCl_3$ (^{19}F = -112.96 ppm)[28] and phenylboronic acid pinacol ester in $CDCl_3$ (^{11}B = 26.67 ppm)[29]. Carbons *ipso* to boron substituents were not observed in ^{13}C NMR due to quadrupolar broadening[30]. IUPAC names were adapted from ChemBioDraw Ultra 15.1 (PerkinElmer). Purities were measured with the aid

of analytical LC−MS using a Shimadzu LC-20AD liquid chromatography pump system with a Shimadzu SPDM20A diode array detector with the MS detection performed with a Shimadzu LC-MS-2010EV mass spectrometer operating in both positive and negative ionization mode. The column used was an Xbridge (C18) 5 µm column (50 mm × 4.6 mm). The following solutions are used for the eluents. Solvent A: water/formic acid 999:1 and solvent B: acetonitrile/formic acid 999:1. The eluent program used is as follows: flow rate: 1.0 mL/min, start 95% A in a linear gradient to 10% A over 4.5 min, hold 1.5 min at 10% A, in 0.5 min in a linear gradient to 95% A, hold 1.5 min at 95% A, total run time: 8.0 min. Compound purities were calculated as the percentage peak area of the analyzed compound by UV detection at 254 nm. Synthesized boronic acid pinacol esters **33**-**48** were found to be unstable under the LC-MS conditions. All chemistry and analyses of photosensitive compounds were carried out under dimmed or red light.

Photochemistry

UV−Vis spectra were recorded using a Thermo-scientific Evolution 201 PC spectrophotometer equipped with a thermostated cell holder set at 20 ^{O}C. Fits of UV−vis spectroscopy data were generated using Mathworks Matlab R2014A (8.3.0.532). Illumination was executed using a Sutter instruments Lambda LS with a 300 W full-spectrum lamp connected to a Sutter instruments Lambda 10-3 optical filter changer equipped with 434 ± 9 nm and 360 ± 20 nm filters. The light intensity used in all illuminations is 0.77 mW/mm^2 using the 360 ± 20 nm filter and 0.57 mW/mm^2 for the 434 ± 9 nm filter as measured using a Thorlabs PM16−401 power meter. For photochemical analyses illuminations were performed in Hellma Suprasil quartz 114- QS cuvettes. Thermal relaxation experiments and Arrhenius extrapolations were performed according to Priimagi et al.[26] using a compound concentration of 25 µM in 50 mM Tris−HCl pH 7.4 buffer +1% DMSO-d_6 and temperatures of 60, 70, and
80 °C. Illuminations for pharmacological experiments were performed in cylindrical clear glass vials with a volume of 4.5 mL.
The typical distance between light source and vial or cuvette was 2 cm. In illuminations during TVEC experiments the light- source was positioned at 5 cm from the chamber containing the oocyte. The focused beam of the light source has a diameter of 1.8 cm and the beam was pointed such that it illuminated the full oocyte.

Cell culture

Human embryonic kidney 293T (HEK293T) cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% FBS, penicillin (50 IU/mL) and streptomycin (50 µg/ml) at 37°C with 5% CO_2.

Transfection and membrane preparation

For [^{35}S]-GTPγS assays, HEK293T cells were transfected with 2500 ng hH_3R cDNA and 2500 ng pcDEF3 cDNA using the PEI method. After 48 h cells were detached from plates and collected in PBS and centrifuged at 1500 g for 5 min. Cell pellets were resuspended in membrane buffer [15 mM Tris-HCl, 1 mM EGTA, 0.3 mM EDTA and 2 mM $MgCl_2$, pH 7.4] and sonicated for 10 sec before centrifugation at 1900 g for 30 min at 4 °C. The membrane pellet was resuspended in 20 mM Tris-HCl with 250 mM sucrose pH 7.4 and aliquots were stored at -80°C until the day of experiment. For radioligand displacement assays, cells were collected as described for GTPγS assay. Subsequently, cells were centrifuged at 1900 g for 10 min and the cell-pellet was stored at -20 °C until day of experiment.

Radioligand binding

Radioligand binding assays were performed as previously reported in Chapter 5. [11] In brief, 1 mM stock of photoswitchable ligand was divided in two samples that were either illuminated at 360 ± 20 nm or kept in the dark to obtain PSS *cis* and *trans* isomer, respectively. [^{3}H]-N-α-methylhistamine (NAMH) binding assays were performed by competing increasing concentrations of cold ligand prepared in assay buffer (50 mM Tris-HCl pH 7.4) with 2 nM [^{3}H]-NAMH (PerkinElmer, Boston, MA, USA, specific activity 79.7 Ci/mmol) for binding to HEK293T cell homogenates transiently expressing the hH_3R, mH_3R or rH_3R in a black 96 well plate. The reaction mixture was incubated for 2 h at 25 °C before termination by rapid filtration over a 96 well GF/C filter plate pre-coated with 0.5% PEI using Perkin Elmer 96-well filtermate-harvester (Perkin Elmer). The filter was washed 5 times with ice-cold wash buffer (50 mM Tris-HCl pH 7.4, 4°C). 5 h after addition of Microscint O scintillation liquid (PerkinElmer) filter-bound radioactivity was measured using a Microbeta Wallac trilux scintillation counter (Perkin Elmer).

Binding Selectivity Screening.

H_1R radioligand binding assays were performed according to the method of Kuhne et al.[31] using an incubation time of 2 h instead of 4 h. H_2R radioligand binding assays were performed according to Leurs et al.[32] using HEK293T cells instead of CHO cells and a total volume of 100 µL instead of 400 µL. H_4R radioligand binding assays were performed according to the method of Nijmeijer et al.[33]

[^{35}S]-GTPγS assay

The [^{35}S]-GTPγS assay was performed in a total volume of 200 µL consisting of increasing concentrations of unlabelled ligand, either kept in the dark or pre-illuminated at 360 ± 20 nm, in GTPγS buffer (50 mM HEPES, 150 mM NaCl, 10 mM $MgCl_2$, 1 µM GDP and 0.02

µg/µL saponin, pH 7.4), 2 nM [^{35}S]-GTPγS (PerkinElmer, Boston, MA, USA, specific activity 2200 Ci/mmol) and 20 µg hH_3R expressing HEK293T cell homogenates. The reaction mixture was incubated for 1 h at 25°C before harvesting over a 96-well GF/B-filter using Perkin Elmer filtermate-harvester (Perkin Elmer) followed by washing with ice-cold wash buffer [Tris-HCl pH 7.4, 4 °C]. 5 h after addition of Microscint O scintillation liquid filter-bound radioactivity was measured using a Microbeta Wallac trilux scintillation counter (Perkin Elmer).

cRNA Synthesis

The Kir3.1 and Kir3.4 were both supplied in the pcDNA3.1 vector and were a kind gift of K. Sahlholm (Karolinska Institute, Stockholm, Sweden). The H_3R was cloned into the pCIneo vector. Restriction enzymes BAMHI and NdeI were used to linearize the constructs pCIneo-H3R and pcDNA3.1-Kir3.1 and 3.4, respectively. After the digestion, 0.5% SDS and Proteinase K (200 µg/mL) were added, and the mixture was incubated at 50 °C for 30 min. DNA was extracted by phenol/chloroform and the precipitation method. The quality and digestion were checked on 1% agarose gel. The cRNA synthesis was performed with the T7 mMessage mMachine kit (Ambion, Austin, TX). After the synthesis, the cRNA quality and amount were checked on a denaturating gel for RNA as described by Almeida et al. (2014) [34].

H_3R and GIRK Expression in Oocytes

Xenopus laevis oocytes were supplied as “topgrade” oocytes (Ecocyte, Castrop-Rauxel, Germany). Each oocyte was injected with a volume of 46 nL, containing 3 ng cRNA of each GIRK subunit and 50 ng cRNA of the H3R or with 46 nL of RNase free water as control with the Nanoject II (Drummond Scientific Company, Broomall, PA). The oocytes were incubated for 4−6 days at 12 °C in modified Barth solution (88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO3, 15 mM HEPES, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 2.5 mg pyruvic acid, 100 µg/mL streptomycin, 50 µg/mL gentamycin, adjusted with Tris to pH 7.4), as described by Sahlholm et al. (2007)[35].

Two-Electrode Voltage Clamp

TEVC was performed with an Axoclamp 900A amplifier and a Digidata 1550 Digitizer. The currents were recorded and analyzed with pClamp 10.6 (Molecular Devices, Sunnyvale, CA). Glass micropipettes were pulled from borosilicate capillaries (GC150-10, Harvard Apparatus, Edenbridge, UK) with the P-1000 micropipette puller (Sutter Instrument, Novato, CA) to have a resistance of 1−3 MΩ when filled with 3 M KCl solution. The oocyte was positioned in the recording chamber (RC-1Z, Warner Instruments, Hamden, CT), and by gravity flow, high potassium solution (64 mM NaCl, 25 mM KCl, 0.8 mM $MgCl_2$, 0.4 mM $CaCl_2$, 15 mM HEPES and adjusted with Bis-Tris Propane to pH 7.4) was perfused

through the recording chamber. For the study of H_3R ligands, the membrane potential was clamped at −80 mV and the evoked currents were measured at room temperature (~20 °C). Ligand **65** was diluted in the high potassium solution to obtain the appropriate concentrations. For photoswitchable ligand **65**, TEVC was performed in the dark, and the different wavelengths (360 ± 20 nm and 343 ± 9 nm) were applied directly on the oocyte in the recording chamber for 1 min with the Sutter instruments Lambda 10-3 optical filter changer equipped with 434 ± 9 nm and 360 ± 20 nm filters.

Data analysis

Data were analyzed using GraphPad Prism 7.02 (Graphpad software Inc, San Diego, USA). Shown data are mean ± S.E.M. of at least 3 independent experiment performed in triplicate unless stated otherwise. Competition binding curves were fitted using non-linear regression to a one-sited binding model and obtained IC_{50} values were converted to k_i values using the Cheng-Prusoff equation[36]. Functional experiments were subjected to sigmoidal dose-response curves using non-linear regression.

Acknowledgements:

We thank Hans Custers for HRMS analyses, Andrea van de Stolpe for setting up the photochemistry equipment and Fons Lefeber (Leiden University) for NMR assistance. Kristoffer Sahlholm (Karolinska institute) is kindly acknowledged for providing the pcDNA3.1-Kir3.1 and -Kir3.4 plasmids.

Supplemental information

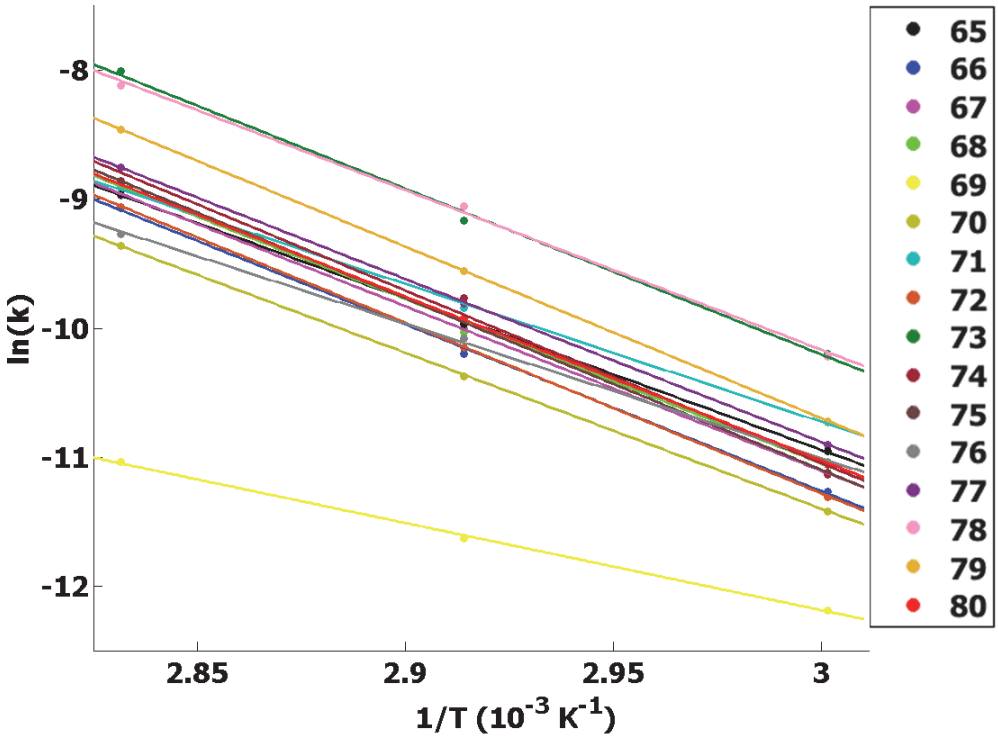


Figure S1: Arrhenius plots for all compounds measured at a compound concentration of 25 µM in 50 mM Tris-HCl pH 7.4 buffer + 1% DMSO-d6. Values in Table 1 are extrapolations of the linear fits presented in this plot. R2 values for all fits were over 0.99.

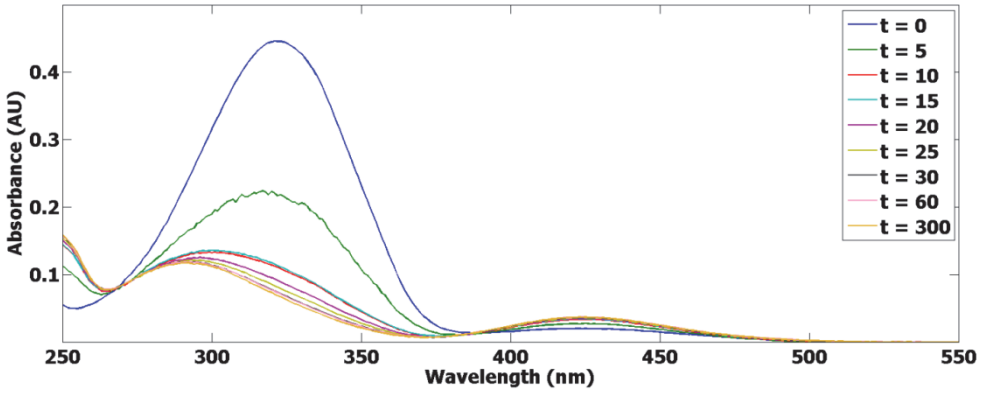


Figure S2: Illumination of 25 µM of 65 (trans) in 50 mM Tris-HCl pH 7.4 buffer containing 1% DMSO-d6 using 360 ± 20 nm and analyzed using UV-vis spectrometry. Value t is in seconds.

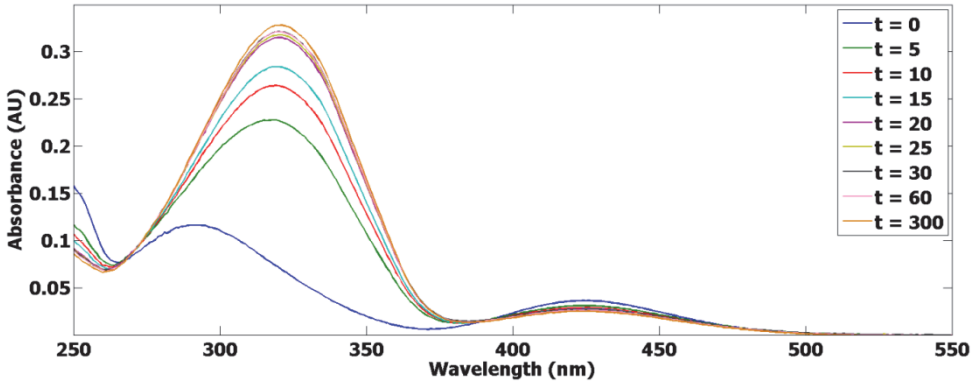


Figure S3: Illumination of 25 µM of 65 (PSS cis, obtained as shown in Figure S2) in 50 mM Tris-HCl pH 7.4 buffer containing 1% DMSO-d6 using 434 ± 9 nm and analyzed using UV-vis spectrometry. Value t is in seconds.

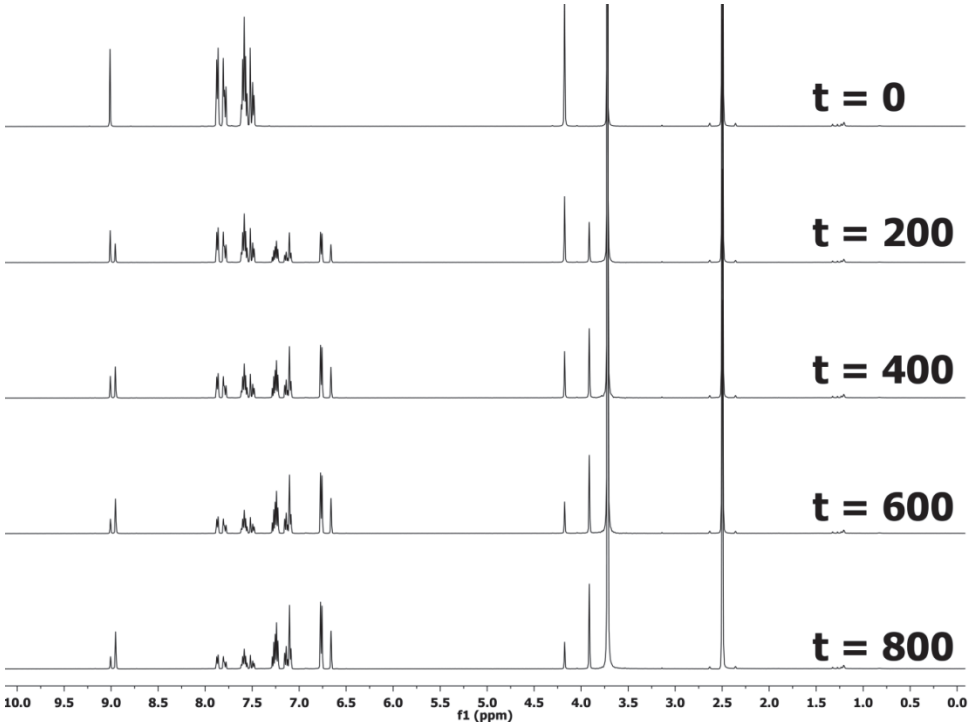


Figure S4: ^{1}H NMR spectra of an illumination with 360 ± 20 nm of 10 mM of compound 65 in DMSO-d_6. The value t is illumination time in seconds.

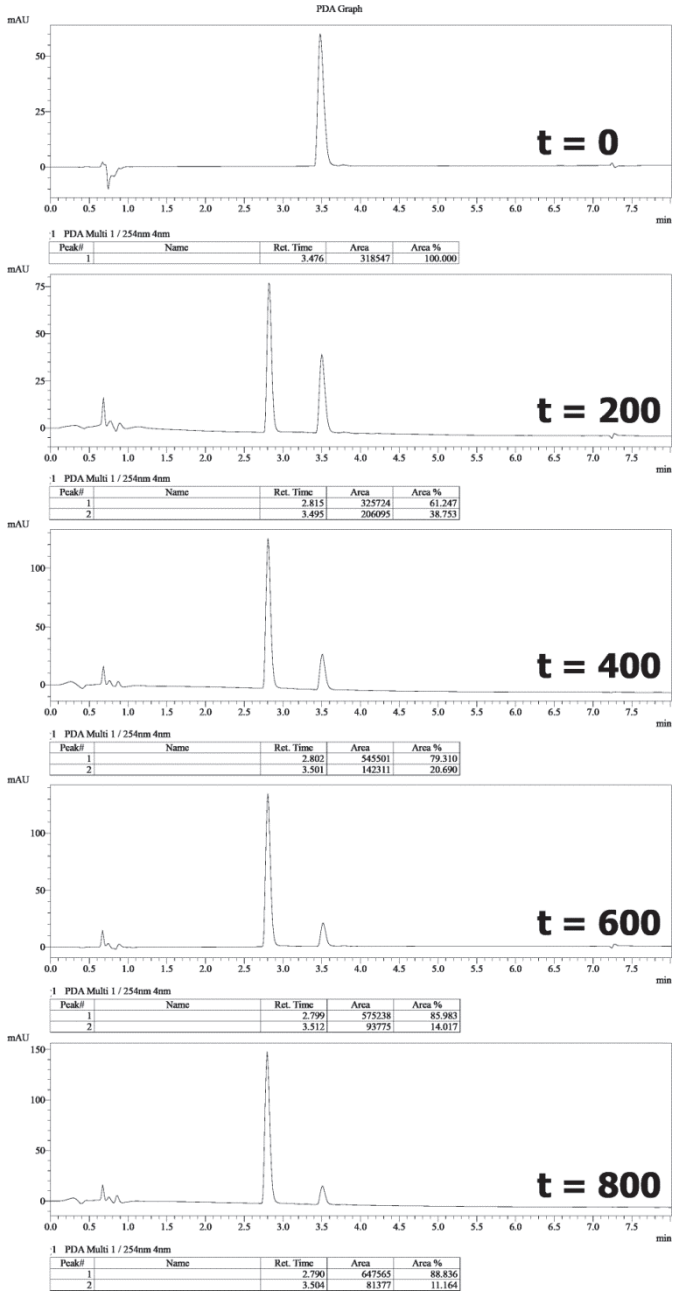


Figure S5: LC chromatograms corresponding to the experiment and 1H NMR spectra shown in Figure S4. The value t is illumination time in seconds.

Table S1. Correlation between ^{1}H NMR and LC-MS area percentages of 10 mM compound in DMSO-d_6 under illumination with 360 ± 20 nm for the indicated duration.

Time (s)	^{1}H NMR		LC-MS	
	%trans	%cis	%trans	%cis
0	>95	<5	>99%	<1
200	61.63	38.37	38.75	61.25
400	40.95	59.05	20.69	79.31
600	30.23	69.77	14.02	85.98
800	25.26	74.74	11.16	88.84

Aliquots were diluted 100 times in Tris-HCl pH 7.4 buffer prior to LC-MS analysis. The photostationary states of the compounds were not reached at any of the chosen time points.

Table S2. Selectivity data of compound 65 on the hH_1R, hH_2R, hH_3R, mH_3R, rH_3R and hH_4R.

	65 (VUF15000)			
	pK_i *trans* ± SEM	pK_i at PSS *cis* ± SEM	pK_i shift	Fold selective
hH_1R	< 5	< 5	N.A.	>300
hH_2R	< 5	< 5	N.A.	>300
hH_3R	8.4 ± 0.0	7.5 ± 0.1	-0.9 ± 0.1	-
mH_3R	6.7 ± 0.0	6.1 ± 0.1	-0.6 ± 0.1	-
rH_3R	7.2 ± 0.1	6.3 ± 0.0	-0.9 ± 0.0	
hH_4R	7.0 ± 0.0	6.0 ± 0.1	-1.0 ± 0.1	30

Displacement of [^{3}H]-mepyramine on hH_1R, [^{125}I]-iodoaminopotentidine on hH_2R, [^{3}H]-N-α-methylhistamine on hH_3R and mH_3R, and rH_3R [^{3}H]-histamine on the hH_4R were used. Data are mean ± S.E.M. of at least 3 experiments performed in triplicate.

Chemical procedures

4-Formyl-*N,N*-dimethyl-1H-imidazole-1-sulfonamide (3)

To a stirred solution of 1H-imidazole-4-carbaldehyde **2** (20.0 g, 208 mmol) in anhydrous DCM (333 mL) and acetonitrile (83 mL) were added consecutively *N,N*-dimethylsulfamoyl chloride (23.6 mL, 219 mmol) and TEA (34.8 mL, 250 mmol) in a dropwise fashion. The mixture was stirred overnight at RT. The reaction mixture was concentrated *in vacuo* after which H_2O (500 mL) was added and the solution was extracted using EtOAc (3x500 mL). The combined organic phases were dried over Na_2SO_4, concentrated *in vacuo* and purified using flash column chromatography eluting with EtOAc:TEA 99:1, to yield the title compound as a white solid (26.4 g, 62%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.95 (s, 1H), 7.96 (d, *J* = 1.1 Hz, 1H), 7.89 (d, *J* = 1.2 Hz, 1H), 2.93 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 185.9, 142.4, 137.6, 122.1, 38.3. LC-MS: t_r= 2.67 min, purity: >99%, M/z $[M+H]^+$ 204.

4-(Hydroxymethyl)-*N,N*-dimethyl-1H-imidazole-1-sulfonamide (4)

To a stirred solution of aldehyde **3** (26.8 g, 132 mmol) and anhydrous MeOH (264 ml) was added $NaBH_4$ (6.23 g, 165 mmol) in a portion wise fashion. The solution was stirred for 2 h at RT, after which it was quenched by pouring it in ice water (500 mL). The mixture was extracted using DCM (3x200 mL). The combined organic phases were dried over Na_2SO_4 and evaporated *in vacuo* to yield the title compound as a white solid (21.2 g, 78%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.08 (d, *J* = 1.2 Hz, 1H), 7.38 (d, *J* = 0.9 Hz, 1H), 5.12 (t, *J* = 5.7 Hz, 1H), 4.39 (d, *J* = 5.6 Hz, 2H), 2.80 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 144.4, 136.7, 114.7, 57.2, 37.8. LC-MS: t_r= 2.67 min, purity: 98.3%, M/z $[M+H]^+$ 206.

4-(Chloromethyl)-*N,N*-dimethyl-1H-imidazole-1-sulfonamide (5)

To an ice-cooled solution of TEA (26.2 ml, 188 mmol) and alcohol **4** (19.3 g, 94.0 mmol) in anhydrous THF (235 mL) were added LiCl (12.0 g, 282 mmol) and methanesulfonyl chloride (8.79 ml, 113 mmol). The solution was stirred for 1 h after which the ice bath was removed and stirring was continued for another 3 h. Water (500 mL) was added and the mixture was extracted using EtOAc (3x500 mL). The combined organic phases were washed once using brine (500 mL), dried over Na_2SO_4 and evaporated *in vacuo* to yield the title compound as a yellowish oil which crystallized upon storage in the freezer (20.5 g, 97%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.18 (d, *J* = 1.4 Hz, 1H), 7.71 (s, 1H), 4.66 (s, 2H), 2.82 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 139.2, 137.4, 117.2, 39.0, 37.8. LC-MS: t_r= 3.32 min, purity: 97.6%, M/z $[M+H]^+$ 224.

General procedure A:
(E)-1-Phenyl-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)diazene (33)

3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline **26** (4.00 g, 18.3 mmol) was added to a solution of nitrosobenzene **16** (1.96 g, 18.3 mmol) in DCM (100 mL). After 5 min, glacial AcOH (100 mL) was added and the solution was stirred overnight at RT. The reaction mixture was concentrated *in vacuo* and subjected to flash column chromatography using a gradient from cyclohexane to EtOAc: cyclohexane 1:9 as eluent. This yielded the title compound as an orange solid (5.42 g, 96%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.01 (ddd, *J* = 7.9, 2.0, 2.0 Hz, 1H), 7.96 – 7.89 (m, 3H), 7.56 – 7.49 (m, 3H), 7.49 – 7.44 (m, 1H), 1.38 (s, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 152.8, 152.2, 137.4, 131.1, 129.4, 129.2, 128.7, 125.5, 123.0, 84.2, 25.1. ^{11}B NMR (128 MHz, $CDCl_3$) δ 30.9.

General procedure B:
(E)-1-(2-Fluorophenyl)-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)diazene (34)

To a stirred solution of Oxone™ (19.1 g, 31.0 mmol) in H_2O (129 mL) was added 2-fluoroaniline **7** (1.50 mL, 15.5 mmol) dissolved in DCM (32 mL). The obtained biphasic solution was stirred vigorously for 3 h after which the layers were separated. DCM (50 mL) was added to the organic phase, which was washed using 10% aq. sodium thiosulfate solution (50 mL), 1 M aq. HCl (50 mL) and aq. satd. $NaHCO_3$ (50 mL). The organic phase was transferred to a flask and aniline **26** (3.74 g,

17.0 mmol) was added. After 5 min glacial AcOH (32 mL) was added and the solution was stirred overnight at RT. The reaction mixture was concentrated *in vacuo* and subjected to flash column chromatography using a gradient from cyclohexane to EtOAc: cyclohexane 1:9 as eluent. This yielded the title compound as an orange solid (3.89 g, 77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.38 (s, 1H), 8.05 – 8.00 (m, 1H), 7.93 (d, *J* = 7.3 Hz, 1H), 7.75 (td, *J* = 7.9, 1.6 Hz, 1H), 7.53 (t, *J* = 7.6 Hz, 1H), 7.48 – 7.42 (m, 1H), 7.30 – 7.24 (m, 1H), 7.22 (t, *J* = 7.7 Hz, 1H), 1.38 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.2 (d, *J* = 257.6 Hz), 152.4, 140.8 (d, *J* = 6.9 Hz), 137.9, 132.5 (d, *J* = 8.4 Hz), 130.4, 128.8, 125.0, 124.4 (d, *J* = 3.8 Hz), 117.9, 117.2 (d, *J* = 19.9 Hz), 84.3, 25.1. ^{11}B NMR (128 MHz, $CDCl_3$) δ 31.8. ^{19}F NMR (235 MHz, $CDCl_3$) δ -124.4.

(E)-1-(3-Fluorophenyl)-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)diazene (35)

This compound was synthesized according to general procedure **B,** using Oxone™ (19.2 g, 31.2 mmol), 3-fluoroaniline **8** (1.50 mL, 15.6 mmol), aniline **26** (4.10 g, 18.7 mmol), DCM (15 mL), H_2O (60 mL) and AcOH (15 mL) as starting materials. This yielded the title compound as an orange solid (3.25 g, 64%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.01 (dt, *J* = 8.0, 1.6 Hz, 1H), 7.93 (d, *J* = 7.2 Hz, 1H), 7.77 (d, *J* = 7.9 Hz, 1H), 7.61 (dt, *J* = 9.8, 2.2 Hz, 1H), 7.54 (t, *J* = 7.6 Hz, 1H), 7.52 – 7.46 (m, 1H), 7.18 (td, *J* = 8.1, 2.3 Hz, 1H), 1.38 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.4 (d, *J* = 247.6 Hz), 154.3 (d, *J* = 7.1 Hz), 151.9, 137.9, 130.4 (d, *J* = 8.5 Hz), 129.5, 128.8, 125.7, 120.7 (d, *J* = 2.8 Hz), 117.8 (d, *J* = 22.0 Hz), 108.2 (d, *J* = 22.9 Hz), 84.3, 25.1. ^{11}B NMR (128 MHz, $CDCl_3$) δ 31.2. ^{19}F NMR (235 MHz, $CDCl_3$) δ -112.1.

(E)-1-(4-Fluorophenyl)-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)diazene (36)

This compound was synthesized according to general procedure **B,** using Oxone™ (13.0 g, 21.1 mmol), 4-fluoroaniline **9** (1.00 mL, 10.6 mmol), aniline **26** (2.78 g, 12.7 mmol), DCM (10 mL), H_2O (40 mL) and AcOH (10 mL) as starting materials. This yielded the title compound as an orange solid (2.71 g, 79%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.33 (t, *J* = 1.5 Hz, 1H), 8.00 – 7.97 (m, 1H), 7.97 – 7.93 (m, 2H), 7.91 (dt, *J* = 7.3, 1.2 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.23 – 7.17 (m, 2H), 1.38 (s, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.5 (d, *J* = 251.9 Hz), 152.1, 149.4 (d, *J* = 2.9 Hz), 137.5, 129.3, 128.7, 125.5, 125.0 (d, *J* = 8.9 Hz), 116.2 (d, *J* = 22.8 Hz), 84.3, 25.1. ^{11}B NMR (128 MHz, $CDCl_3$) δ 31.3. ^{19}F NMR (235 MHz, $CDCl_3$) δ -109.5.

(E)-1-(2,6-Difluorophenyl)-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)diazene (37)

This compound was synthesized according to general procedure **B,** using Oxone™ (11.4 g, 18.6 mmol), 2,6-difluoroaniline **10** (1.00 mL, 9.29 mmol), aniline **26** (2.04 g, 9.29 mmol), DCM (21 mL), H_2O (85 mL) and AcOH (21 mL) as starting materials. The title compound was obtained as an orange solid (2.56 g, 80%). ^{1}H NMR (600 MHz $CDCl_3$) δ 8.37 (s, 1H), 8.00 (d, *J* = 7.8 Hz, 1H), 7.95 (d, *J* = 7.2 Hz, 1H), 7.53 (t, *J* = 7.6 Hz, 1H), 7.31 (p, *J* = 7.8 Hz, 1H), 7.04 (t, *J* = 8.8 Hz, 2H), 1.37 (s, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.9 (dd, *J* = 259.2, 4.4 Hz), 152.8, 138.4, 131.7 (t, *J* = 10.2 Hz), 130.2 (t, *J* = 10.3 Hz), 130.0, 128.7, 112.6 (dd, *J* = 20.0, 4.1 Hz), 84.3, 25.1. ^{11}B NMR (128 MHz, $CDCl_3$) δ 30.9. ^{19}F NMR (235 MHz, $CDCl_3$) δ -121.7.

(E)-1-(2-Chlorophenyl)-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)diazene (38)

This compound was synthesized according to general procedure **B,** using Oxone™ (5.61 g, 9.13 mmol), 2-chloroaniline **11** (0.582 g, 4.56 mmol), aniline **26** (1.00 g, 4.56 mmol), DCM (15 mL), H_2O (61 mL), AcOH (15 mL) as starting materials. This yielded the title compound as a red solid (0.953 g, 61%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.06 – 8.01 (m, 1H), 7.96 – 7.92 (m, 1H), 7.71 – 7.66 (m, 1H), 7.58 – 7.54 (m, 1H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.42 – 7.37 (m, 1H), 7.36 – 7.32 (m, 1H), 1.38 (s, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 152.4, 149.0, 138.0, 135.3, 131.7, 131.5, 130.8, 128.8, 127.4, 124.4, 117.8, 84.3, 25.1. ^{11}B NMR (128 MHz, $CDCl_3$) δ 30.8.

(E)-1-(4-Chlorophenyl)-2-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)diazene (39)

This compound was synthesized according to general procedure **B,** using Oxone™ (5.61 g, 9.13 mmol), 4-chloroaniline **12** (0.582 g, 4.56 mmol), aniline **26** (1.00 g, 4.56 mmol), DCM (15 mL), H_2O (61 mL), AcOH (15 mL) as starting materials. This yielded the title compound as a red solid (1.02 g, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.02 – 7.97 (m, 1H), 7.94 – 7.91 (m, 1H), 7.88 (d, *J* = 8.1 Hz, 2H), 7.53 (t, *J* = 7.6 Hz, 1H), 7.49 (d, *J* = 8.1 Hz, 2H), 1.38 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.0, 151.1, 137.7, 137.0, 129.5, 129.4, 128.8, 125.6, 124.3, 84.3, 25.1. ^{11}B NMR (128 MHz, $CDCl_3$) δ 30.3.

(E)-1-(2-Methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-2-phenyldiazene (40)

This compound was synthesized according to general procedure **A,** using 2-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline **27** (1.02 g, 4.39 mmol), nitrosobenzene **16** (0.471 g, 4.39 mmol), DCM (50 mL) and AcOH (50 mL) as starting materials. The title compound was obtained as an orange solid (1.03 g, 73%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.92 (d, *J* = 7.3 Hz, 2H), 7.87 (d, *J* = 7.2 Hz, 1H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.52 (t, *J* = 7.5 Hz, 2H), 7.46 (t, *J* = 7.3 Hz, 1H), 7.28 – 7.23 (m, 1H), 2.92 (s, 3H), 1.39 (s, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.2, 151.0, 144.7, 138.8, 130.8, 129.2, 125.7, 123.1, 118.2, 83.9, 25.1, 16.4. ^{11}B NMR (128 MHz, $CDCl_3$) δ 32.0.

(E)-1-(4-Methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-2-phenyldiazene (41)

This compound was synthesized according to general procedure **A,** using 4-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline **28** (0.500 g, 2.15 mmol), nitrosobenzene **16** (0.230 g, 2.15 mmol), DCM (15 mL) and AcOH (15 mL) as starting materials. The title compound was obtained as an orange solid (0.521 g, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (d, *J* = 2.3 Hz, 1H), 7.94 – 7.89 (m, 2H), 7.86 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.53 – 7.48 (m, 2H), 7.48 – 7.42 (m, 1H), 7.31 (d, *J* = 8.2 Hz, 1H), 2.62 (s, 3H), 1.38 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.9, 150.2, 148.7, 131.7, 130.9, 130.7, 129.2, 123.9, 122.9, 83.9, 25.1, 22.4. ^{11}B NMR (128 MHz, $CDCl_3$) δ 31.3.

(E)-1-(3-Methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-2-phenyldiazene (42)

This compound was synthesized according to general procedure **A,** using 3-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline **29** (2.60 g, 11.2 mmol), nitrosobenzene **16** (1.20 g, 11.2 mmol), DCM (75 mL) and AcOH (75 mL) as starting materials. The title compound was obtained as an orange solid (2.41 g, 67%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.91 (d, *J* = 7.7 Hz, 2H), 7.81 (s, 1H), 7.75 (s, 1H), 7.51 (t, *J* = 7.4 Hz, 2H), 7.46 (t, *J* = 7.5 Hz, 1H), 2.46 (s, 3H), 1.38 (s, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 152.9, 152.4, 138.6, 138.2, 131.0, 129.2, 127.1, 125.7, 122.9, 84.2, 25.1, 21.3. ^{11}B NMR (128 MHz, $CDCl_3$) δ 30.9.

(E)-1-(2-Methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-2-phenyldiazene (43)

This compound was synthesized according to general procedure **A,** using 2-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline **30** (1.69 g, 7.26 mmol), nitrosobenzene **16** (0.777 g, 7.26 mmol), DCM (75 mL) and AcOH (75 mL) as starting materials. The title compound was obtained as an orange solid (1.89 g, 81%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.96 – 7.91 (m, 2H), 7.80 (dd, *J* = 7.5, 1.3 Hz, 1H), 7.54 – 7.49 (m, 2H), 7.48 – 7.44 (m, 1H), 7.35 (d, *J* = 7.5 Hz, 1H), 2.73 (s, 3H), 1.36 (s, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.2, 150.6, 141.3, 137.2, 130.9, 130.9, 129.2, 123.1, 121.8, 84.0, 25.0, 18.0. ^{11}B NMR (128 MHz, $CDCl_3$) δ 31.2.

(E)-1-(3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-2-(o-tolyl)diazene (44)

This compound was synthesized according to general procedure **B,** using Oxone™ (5.61 g, 9.13 mmol), *o*-toluidine **13** (0.489 g, 4.56 mmol), aniline **26** (1.00 g, 4.56 mmol), H_2O (60.9 mL), DCM (15.2 mL) and AcOH (15.2 mL) as starting materials. This yielded the title compound as a yellow solid (801 mg, 55%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.00 – 7.96 (m, 1H), 7.91 (d, *J* = 7.3 Hz, 1H), 7.62 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.51 (t, *J* = 7.6 Hz, 1H), 7.36 – 7.33 (m, 2H), 7.28 – 7.24 (m, 1H), 2.74 (s, 3H), 1.38 (s, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 152.6, 151.0, 138.2, 137.2, 131.4, 130.9, 130.6, 128.7, 126.5, 124.5, 115.7, 84.2, 25.1, 17.7. ^{11}B NMR (128 MHz, $CDCl_3$) δ 31.8.

(E)-1-(3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-2-(m-tolyl)diazene (45)

This compound was synthesized according to general procedure **B,** using Oxone™ (5.61 g, 9.13 mmol), *m*-toluidine **14** (0.489 g, 4.56 mmol), aniline **26** (1.00 g, 4.56 mmol), H_2O (60.9 mL), DCM (15.2 mL) and AcOH (15.2 mL) as starting materials. This yielded the title compound as an orange solid (827 mg, 59%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 – 8.32 (m, 1H), 7.99 (app. dt, *J* = 8.0, 1.7 Hz, 1H), 7.94 – 7.88 (m, 1H), 7.77 – 7.71 (m, 2H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.40 (t, *J* = 7.9 Hz, 1H), 7.29 (d, *J* = 7.5 Hz, 1H), 2.46 (s, 3H), 1.38 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.9, 152.2, 139.1, 137.3, 131.9, 129.3, 129.0, 128.7, 125.5, 123.1, 120.7, 84.2, 25.1, 21.5. ^{11}B NMR (128 MHz, $CDCl_3$) δ 30.6.

(E)-1-(3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-2-(p-tolyl)diazene (46)

This compound was synthesized according to general procedure **B**, using Oxone™ (5.61 g, 9.13 mmol), *p*-toluidine **15** (0.489 g, 4.56 mmol), aniline **26** (1.00 g, 4.56 mmol), H_2O (60.9 mL), DCM (15.2 mL) and AcOH (15.2 mL) as starting materials. This yielded the title compound as an orange solid (927 mg, 63%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.98 (app. dt, *J* = 8.0, 1.6 Hz, 1H), 7.89 (app. dt, *J* = 7.4, 1.4 Hz, 1H), 7.86 – 7.82 (m, 2H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 2H), 2.44 (s, 3H), 1.37 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.3, 150.9, 141.6, 137.1, 129.9, 129.2, 128.7, 125.4, 123.0, 84.2, 25.1, 21.7. ^{11}B NMR (128 MHz, $CDCl_3$) δ 30.4.

(E)-1-(4-Fluoro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-2-phenyldiazene (47)

This compound was synthesized according to general procedure **A,** using 2-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline **31** (750 mg, 3.16 mmol), nitrosobenzene **16** (0.339 g, 3.16 mmol), DCM (32 mL) and AcOH (32 mL) as starting materials. The title compound was obtained as an orange solid (492 mg, 48%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 – 8.14 (m, 1H), 7.99 – 7.94 (m, 2H), 7.93 – 7.87 (m, 1H), 7.57 – 7.45 (m, 3H), 7.29 – 7.23 (m, 1H), 1.36 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.2 (d, *J* = 261.6 Hz), 153.0, 140.4 (d, *J* = 6.5 Hz), 139.2 (d, *J* = 8.8 Hz), 131.5, 129.2, 124.5, 123.3, 116.7 (d, *J* = 19.2 Hz), 84.3, 25.0. ^{11}B NMR (128 MHz, $CDCl_3$) δ 31.7. ^{19}F NMR (471 MHz, $CDCl_3$) δ -120.1.

(E)-1-(2-Fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-2-phenyldiazene (48)

This compound was synthesized according to general procedure **A,** using 4-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline **32** (750 mg, 3.16 mmol), nitrosobenzene **16** (0.342 g, 3.20 mmol), DCM (32 mL) and AcOH (32 mL) as starting materials. The title compound was obtained as an orange solid (621 mg, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (dd, *J* = 5.5, 2.7 Hz, 1H), 8.07 – 7.98 (m, 1H), 7.95 – 7.88 (m, 2H), 7.56 – 7.46 (m, 3H), 7.18 (t, *J* = 8.6 Hz, 1H), 1.40 (s, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.8 (d, *J* = 257.1 Hz), 152.7, 148.8 (d, *J* = 3.1 Hz), 132.7 (d, *J* = 9.3 Hz), 131.1, 129.2, 127.0 (d, *J* = 9.9 Hz), 122.9, 116.4 (d, *J* = 25.9 Hz), 84.4, 25.0. ^{11}B NMR (128 MHz, $CDCl_3$) δ 30.3. ^{19}F NMR (471 MHz, $CDCl_3$) δ -98.9.

General procedure C

(E)-*N,N*-Dimethyl-4-(3-(phenyldiazenyl)benzyl)-1H-imidazole-1-sulfonamide (49)

A stirred mixture of chloride **5** (336 mg, 1.50 mmol), Na_2CO_3 (334 mg, 3.15 mmol) and boronic acid pinacol ester **33** (462 mg, 1.50 mmol) in a mixture of H_2O (3.0 ml) and DME (6.0 ml) was degassed by purging with N_2 for 5 min. $Pd(PPh_3)_4$ (87 mg, 0.075 mmol) was added and the solution was sealed with a septum. The mixture was heated for 1 h at 130 °C in the microwave. The mixture was diluted using H_2O (10 mL) and extracted using EtOAc (3x15 mL). The combined organic phases were dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was further purified by flash column chromatography using a gradient from cyclohexane: EtOAc 2:8 to EtOAc. The title compound was obtained as an orange oil (0.47 g, 85%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 – 7.86 (m, 3H), 7.83 – 7.78 (m, 2H), 7.55 – 7.49 (m, 2H), 7.49 – 7.44 (m, 2H), 7.41 (d, *J* = 7.7 Hz, 1H), 6.93 (s, 1H), 4.05 (s, 2H), 2.83 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.0, 152.7, 143.5, 139.9, 136.7, 131.6, 131.2, 129.5, 129.2, 123.1, 122.9, 121.6, 114.6, 38.3, 34.7. LC-MS: t_r= 5.01 min, purity: >99%, M/z $[M+H]^+$ 370.

(E)-4-(3-((2-Fluorophenyl)diazenyl)benzyl)-*N,N*-dimethyl-1H-imidazole-1-sulfonamide (50)

This compound was synthesized according to general procedure **C,** using chloride **5** (0.411 g, 1.84 mmol), Na_2CO_3 (342 mg, 3.22 mmol), boronic acid pinacol ester **34** (0.500 g, 1.53 mmol), $Pd(PPh_3)_4$ (89 mg, 0.077 mmol), H_2O (6.0 mL) and DME (12 mL) as starting materials. This yielded the title compound as an orange solid (0.49 g, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.87 – 7.82 (m, 1H), 7.81 (s, 1H), 7.74 (td, *J* = 7.9, 1.6 Hz, 1H), 7.52 – 7.41 (m, 3H), 7.31 – 7.20 (m, 2H), 6.93 (s, 1H), 4.05 (s, 2H), 2.84 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.1 (d, *J* = 257.3 Hz), 153.0, 143.2, 140.6 (d, *J* = 6.9 Hz), 139.9, 136.6, 132.6 (d, *J* = 8.4 Hz), 132.1, 129.4, 124.4 (d, *J* = 3.7 Hz), 122.5 (d, *J* = 6.3 Hz), 117.7, 117.0 (d, *J* = 19.9 Hz), 114.5, 38.2, 34.5. ^{19}F NMR (471 MHz, $CDCl_3$) δ -124.5. LC-MS: t_r= 5.01 min, purity: 97.0%, M/z $[M+H]^+$ 388.

(E)-4-(3-((3-Fluorophenyl)diazenyl)benzyl)-*N,N*-dimethyl-1H-imidazole-1-sulfonamide (51)

This compound was synthesized according to general procedure **C,** using chloride **5** (823 mg, 3.68 mmol), Na_2CO_3 (682 mg, 6.44 mmol), boronic acid pinacol ester **35** (1.00 g, 3.07 mmol), $Pd(PPh_3)_4$ (177 mg, 0.153 mmol), H_2O (6.0 mL) and DME (12 mL) as starting materials. This yielded the title compound as an orange solid (0.725 g, 61%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.84 – 7.79 (m, 2H), 7.77 – 7.72 (m, 1H), 7.59 (dt, *J* = 9.8, 2.2 Hz, 1H), 7.53 – 7.46 (m, 2H), 7.43 (d, *J* = 7.5 Hz, 1H), 7.18 (td, *J* = 8.2, 2.4 Hz, 1H), 6.93 (s, 1H), 4.05 (s, 2H), 2.84 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.4 (d, *J* = 247.7 Hz), 154.2 (d, *J* = 7.0 Hz), 152.8, 143.3, 140.0, 136.7, 132.2, 130.4 (d, *J* = 8.4 Hz), 129.6, 123.3, 121.8, 120.6 (d, *J* = 2.9 Hz), 117.9 (d, *J* = 22.0 Hz), 114.6, 108.2 (d, *J* = 22.9 Hz), 38.4, 34.6. ^{19}F NMR (235 MHz, $CDCl_3$) δ -112.1. LC-MS: t_r= 5.27 min, purity: >99%, M/z $[M+H]^+$ 388.

(E)-4-(3-((4-Fluorophenyl)diazenyl)benzyl)-*N,N*-dimethyl-1H-imidazole-1-sulfonamide (52)

This compound was synthesized according to general procedure **C,** using chloride **5** (272 mg, 1.21 mmol), Na_2CO_3 (225 mg, 2.13 mmol), boronic acid pinacol ester **36** (330 mg, 1.01 mmol), $Pd(PPh_3)_4$ (58 mg, 0.051 mmol), H_2O (2.0 mL) and DME (3.9 mL) as starting materials. This yielded the title compound as an orange solid (0.26 g, 67%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 – 7.90 (m, 2H), 7.87 (d, *J* = 1.1 Hz, 1H), 7.82 – 7.77 (m, 2H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.40 (dt, *J* = 7.6, 1.4 Hz, 1H), 7.22 – 7.16 (m, 2H), 6.92 (s, 1H), 4.04 (s, 2H), 2.84 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.5 (d, *J* = 252.0 Hz), 152.8, 149.2 (d, *J* = 3.1 Hz), 143.5, 140.0, 136.8, 131.7, 129.5, 125.0 (d, *J* = 8.9 Hz), 123.1, 121.5, 116.2 (d, *J* = 22.8 Hz), 114.5, 38.4, 34.7. ^{19}F NMR (471 MHz, $CDCl_3$) δ -111.9. LC-MS: t_r= 5.17 min, purity: >99%, M/z $[M+H]^+$ 388.

(E)-4-(3-((2,6-Difluorophenyl)diazenyl)benzyl)-*N,N*-dimethyl-1H-imidazole-1-sulfonamide (53)

This compound was synthesized according to general procedure **C,** using chloride **5** (650 mg, 2.91 mmol), Na_2CO_3 (647 mg, 6.10 mmol), boronic acid pinacol ester **37** (1.00 g, 3.07 mmol), $Pd(PPh_3)_4$ (168 mg, 0.145 mmol), H_2O (6.0 mL) and DME (12 mL) as starting materials. This yielded the title compound as an orange solid (0.888 g, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.82 (d, *J* = 7.7 Hz, 1H), 7.78 (s, 1H), 7.51 – 7.44 (m, 2H), 7.34 – 7.28 (m, 1H), 7.03 (t, *J* = 8.6 Hz, 2H), 6.94 (s, 1H), 4.04 (s, 2H), 2.83 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.8 (dd, J = 258.7, 4.4 Hz), 153.5, 143.3, 140.1, 136.8, 132.7, 131.5 (t, J = 10.2 Hz), 130.4 (t, J = 10.3 Hz), 129.5, 122.7, 122.0, 114.6, 112.6 (dd, J = 20.0, 4.1 Hz), 38.3, 34.6. ^{19}F NMR (471 MHz, $CDCl_3$) δ -121.6. LC-MS: t_r= 4.99 min, purity: 97.1%, M/z $[M+H]^+$ 406.

(E)-4-(3-((2-Chlorophenyl)diazenyl)benzyl)-*N,N*-dimethyl-1H-imidazole-1-sulfonamide (54)

This compound was synthesized according to general procedure **C** using chloride **5** (707 mg, 3.16 mmol), Na_2CO_3 (587 mg, 5.53 mmol), boronic acid pinacol ester **38** (0.903 g, 2.64 mmol), $Pd(PPh_3)_4$ (152 mg, 0.132 mmol), H_2O (5.1 mL) and DME (10 mL) as starting materials. This yielded the title compound as a red oil (0.452 g, 43%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 – 7.83 (m, 3H), 7.70 – 7.65 (m, 1H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.43 (d, *J* = 7.5 Hz, 1H), 7.41 – 7.36 (m, 1H), 7.34 (t, *J* = 7.5 Hz, 1H), 6.94 (s, 1H), 4.05 (s, 2H), 2.83 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.1, 148.8, 143.4, 140.1, 136.8, 135.3, 132.2, 131.8, 130.8, 129.5, 127.4, 123.4, 122.2, 117.7, 114.6, 38.3, 34.7. LC-MS: t_r= 5.39 min, purity: >99%, M/z $[M+H]^+$ 404.

(E)-4-(3-((4-Chlorophenyl)diazenyl)benzyl)-*N,N*-dimethyl-1H-imidazole-1-sulfonamide (55)

This compound was synthesized according to general procedure **C,** using chloride **5** (548 mg, 2.45 mmol), Na_2CO_3 (455 mg, 4.29 mmol), boronic acid pinacol ester **39** (0.700 g, 2.04 mmol), $Pd(PPh_3)_4$ (113 mg, 0.102 mmol), H_2O (3.9 mL) and DME (7.9 mL) as starting materials. This yielded the title compound as a red solid (0.718 g, 87%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.89 – 7.83 (m, 3H), 7.82 – 7.78 (m, 2H), 7.50 – 7.45 (m, 3H), 7.41 (d, *J* = 7.5 Hz, 1H), 6.92 (s, 1H), 4.04 (s, 2H), 2.84 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 152.9, 151.1, 143.4, 140.0, 137.1, 136.8, 131.9, 129.6, 129.5, 124.2, 123.2, 121.6, 114.6, 38.4, 34.7. LC-MS: t_r= 5.36 min, purity: >99%, M/z $[M+H]^+$ 404.

(E)-*N,N*-Dimethyl-4-(2-methyl-3-(phenyldiazenyl)benzyl)-1H-imidazole-1-sulfonamide (56)

This compound was synthesized according to general procedure **C,** using chloride **5** (541 mg, 2.42 mmol), Na_2CO_3 (449 mg, 4.24 mmol), boronic acid pinacol ester **40** (650 mg, 2.02 mmol), $Pd(PPh_3)_4$ (117 mg, 0.101 mmol), H_2O (3.9 mL) and DME (7.8 mL) as starting materials. This yielded the title compound as an orange solid (0.604 g, 78%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.92 (d, *J* = 7.7 Hz, 2H), 7.88 (s, 1H), 7.58 – 7.44 (m, 4H), 7.34 (d, *J* = 7.4 Hz, 1H), 7.28 – 7.22 (m, 1H), 6.77 (s, 1H), 4.07 (s, 2H), 2.81 (s, 6H), 2.67 (s, 3H). A multiplet at 7.28 - 7.22 ppm is obscured by the $CDCl_3$ peak. ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.1, 151.5, 143.4, 138.7, 136.9, 132.6, 131.0, 129.2, 126.5, 123.1, 114.6, 114.5, 38.4, 32.8, 13.3. HSQC analysis indicates the presence of an additional carbon at 136.6 ppm. LC-MS: t_r= 5.62 min, purity: 97.2%, M/z $[M+H]^+$ 384.

(E)-*N,N*-Dimethyl-4-(2-methyl-5-(phenyldiazenyl)benzyl)-1H-imidazole-1-sulfonamide (57)

This compound was synthesized according to general procedure **C,** using chloride **5** (451 mg, 2.02 mmol), Na_2CO_3 (345 mg, 3.26 mmol), boronic acid pinacol ester **41** (500 mg, 1.55 mmol), $Pd(PPh_3)_4$ (90 mg, 0.078 mmol), H_2O (3.0 mL) and DME (6.0 mL) as starting materials. This yielded the title compound as an orange oil (0.35 g, 59%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.90 – 7.85 (m, 2H), 7.79 – 7.74 (m, 2H), 7.54 – 7.43 (m, 3H), 7.36 – 7.32 (m, 1H), 6.79 – 6.76 (m, 1H), 4.06 (s, 2H), 2.83 (s, 6H), 2.37 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.8, 151.4, 142.6, 140.3, 137.6, 131.4, 131.0,

129.2, 124.2, 122.8, 121.7, 114.7, 38.4, 32.5, 19.8. HSQC analysis indicates the presence of an additional carbon at 136.3 ppm. LC-MS: t_r= 5.22 min, purity: >99%, M/z $[M+H]^+$ 384.

(E)-*N,N*-Dimethyl-4-(3-methyl-5-(phenyldiazenyl)benzyl)-1H-imidazole-1-sulfonamide (58)

This compound was synthesized according to general procedure **C,** using chloride **5** (1.25 g, 5.59 mmol), Na_2CO_3 (1.04 g, 9.78 mmol), boronic acid pinacol ester **42** (1.50 g, 4.66 mmol), $Pd(PPh_3)_4$ (269 mg, 0.233 mmol), H_2O (9.0 mL) and DME (17.9 mL) as starting materials. This yielded the title compound as an orange solid (1.49 g, 83%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 – 7.86 (m, 2H), 7.86 (d, *J* = 1.3 Hz, 1H), 7.62 (app. d, *J* = 4.6 Hz, 2H), 7.54 – 7.48 (m, 2H), 7.50 – 7.44 (m, 1H), 7.25 – 7.21 (m, 1H), 6.95 – 6.91 (m, 1H), 4.00 (s, 2H), 2.84 (s, 6H), 2.44 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.2, 152.8, 143.7, 139.8, 139.4, 136.8, 132.5, 131.1, 129.2, 122.9, 121.8, 120.8, 114.5, 38.4, 34.7, 21.5. LC-MS: t_r= 5.25 min, purity: >99%, M/z $[M+H]^+$ 384.

(E)-*N,N*-Dimethyl-4-(4-methyl-3-(phenyldiazenyl)benzyl)-1H-imidazole-1-sulfonamide (59)

This compound was synthesized according to general procedure **C,** using chloride **5** (891 mg, 3.98 mmol), Na_2CO_3 (739 mg, 6.97 mmol), boronic acid pinacol ester **43** (1.07 g, 3.32 mmol), $Pd(PPh_3)_4$ (192 mg, 0.166 mmol), H_2O (6.4 mL) and DME (12.8 mL) as starting materials. This yielded the title compound as an orange solid (0.902 g, 71%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.92 – 7.86 (m, 2H), 7.84 (s, 1H), 7.55 – 7.48 (m, 3H), 7.49 – 7.43 (m, 1H), 7.30 (s, 2H), 6.88 (s, 1H), 3.96 (s, 2H), 2.82 (s, 6H), 2.70 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.0, 150.7, 143.8, 137.0, 136.5, 131.5, 131.5, 130.8, 129.1, 122.9, 115.7, 114.3, 38.2, 34.4, 17.2. HSQC analysis indicates the presence of an additional carbon at 136.5 ppm. LC-MS: t_r= 5.22 min, purity: >99%, M/z $[M+H]^+$ 384.

(E)-*N,N*-Dimethyl-4-(3-(*o*-tolyldiazenyl)benzyl)-1H-imidazole-1-sulfonamide (60)

This compound was synthesized according to general procedure **C,** using chloride **5** (667 mg, 2.98 mmol), Na_2CO_3 (553 mg, 5.22 mmol), boronic acid pinacol ester **44** (801 mg, 2.49 mmol), $Pd(PPh_3)_4$ (144 mg, 0.124 mmol), H_2O (4.8 mL) and DME (9.6 mL) as starting materials. This yielded the title compound as an orange oil (0.645 g, 68%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.83 – 7.77 (m, 2H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.40 (d, *J* = 7.8 Hz, 1H), 7.38 – 7.31 (m, 2H), 7.29 – 7.23 (m, 1H), 6.92 (s, 1H), 4.05 (s, 2H), 2.83 (s, 6H), 2.71 (s, 3H). A multiplet at 7.29 - 7.23 ppm is obscured by the $CDCl_3$ peak. ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.4, 150.9, 143.6, 139.9, 138.2, 136.8, 131.5, 131.4, 131.1, 129.5, 126.6, 123.7, 121.3, 115.6, 114.6, 38.4, 34.8, 17.7. LC-MS: t_r= 5.25 min, purity: 98.6%, M/z $[M+H]^+$ 384.

(E)-*N,N*-Dimethyl-4-(3-(*m*-tolyldiazenyl)benzyl)-1H-imidazole-1-sulfonamide (61)

This compound was synthesized according to general procedure **C,** using chloride **5** (275 mg, 1.23 mmol), Na_2CO_3 (228 mg, 2.15 mmol), boronic acid pinacol ester **45** (330 mg, 1.02 mmol), $Pd(PPh_3)_4$ (59 mg, 0.051 mmol), H_2O (2.0 mL) and DME (4.0 mL) as starting materials. This yielded the title compound as an orange oil (0.27 g, 69%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.83 – 7.77 (m, 2H), 7.73 – 7.69 (m, 2H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.29 (d, *J* = 7.6 Hz, 1H), 6.92 (s, 1H), 4.04 (s, 2H), 2.83 (s, 6H), 2.46 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.1, 152.9, 143.6, 140.0, 139.1, 136.8, 132.0, 131.6, 129.5, 129.1, 123.1, 123.0, 121.5, 120.6, 114.6, 38.4, 34.7, 21.5. LC-MS: t_r= 5.24 min, purity: >99%, M/z $[M+H]^+$ 384.

(E)-*N,N*-Dimethyl-4-(3-(*p*-tolyldiazenyl)benzyl)-1H-imidazole-1-sulfonamide (62)

This compound was synthesized according to general procedure **C,** using chloride **5** (275 mg, 1.23 mmol), Na_2CO_3 (228 mg, 2.15 mmol), boronic acid pinacol ester **46** (330 mg, 1.02 mmol), $Pd(PPh_3)_4$ (59 mg, 0.051 mmol), H_2O (2.0 mL) and DME (4.0 mL) as starting materials. This yielded the title compound as an orange solid (0.26 g, 67%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.83 – 7.76 (m, 4H), 7.46 (t, *J* = 7.7 Hz, 1H), 7.39 (d, *J* = 7.4 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 2H), 6.91 (s, 1H), 4.04 (s, 2H), 2.83 (s, 6H), 2.44 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.1, 150.9, 143.6, 141.8, 139.9, 136.7, 131.4, 129.9, 129.5, 123.0, 121.5, 114.6, 38.4, 34.7, 21.7. HSQC analysis indicates the presence of an additional carbon at 136.0 ppm. LC-MS: t_r= 5.23 min, purity: 95.8%, M/z $[M+H]^+$ 384.

(E)-4-(2-Fluoro-5-(phenyldiazenyl)benzyl)-*N,N*-dimethyl-1H-imidazole-1-sulfonamide (63)

This compound was synthesized according to general procedure **C,** using chloride **5** (760 mg, 3.40 mmol), Na_2CO_3 (630 mg, 5.94 mmol), boronic acid pinacol ester **47** (0.923 g, 2.83 mmol), $Pd(PPh_3)_4$ (163 mg, 0.141 mmol), H_2O (5.5 mL) and DME (11 mL) as starting materials. This yielded the title compound as an orange oil (0.846 g, 77%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 – 7.81 (m, 5H), 7.54 – 7.48 (m, 2H), 7.48 – 7.44 (m, 1H), 7.19 (t, *J* = 8.9 Hz, 1H), 6.99 (s, 1H), 4.05 (s, 2H), 2.83 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.7 (d, *J* = 252.1 Hz), 152.6, 149.2 (d, *J* = 3.2 Hz), 142.0, 136.7, 131.2, 129.2, 126.9 (d, *J* = 17.5 Hz), 125.7 (d, *J* = 7.0 Hz), 123.6 (d, *J* = 9.0 Hz), 122.9, 116.3 (d, *J* = 23.7 Hz), 114.6, 38.3, 28.2 (d, *J* = 3.0 Hz). ^{19}F NMR (471 MHz, $CDCl_3$) δ -113.9. LC-MS: t_r= 5.09 min, purity: >99%, M/z $[M+H]^+$ 388.

(E)-4-(4-Fluoro-3-(phenyldiazenyl)benzyl)-*N,N*-dimethyl-1H-imidazole-1-sulfonamide (64)

This compound was synthesized according to general procedure **C,** using chloride **5** (405 mg, 1.81 mmol), Na_2CO_3 (336 mg, 3.17 mmol), boronic acid pinacol ester **48** (0.492 g, 1.51 mmol), $Pd(PPh_3)_4$ (87 mg, 0.075 mmol), H_2O (2.9 mL) and DME (5.8 mL) as starting materials. This yielded the title compound as an orange solid (0.47 g, 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 – 7.89 (m, 2H), 7.86 (s, 1H), 7.68 – 7.62 (m, 1H), 7.56 – 7.45 (m, 3H), 7.41 – 7.35 (m, 1H), 7.25 – 7.18 (m, 1H), 6.92 (s, 1H), 3.97 (s, 2H), 2.84 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.2 (d, *J* = 256.9 Hz), 152.9, 143.3, 140.5 (d, *J* = 7.1 Hz), 135.1 (d, *J* = 3.7 Hz), 133.0 (d, *J* = 8.2 Hz), 131.6, 129.3, 123.2, 117.9, 117.4 (d, *J* = 20.2 Hz), 114.5, 38.4, 34.1. HSQC analysis indicates the presence of an additional carbon at 136.7 ppm. ^{19}F NMR (471 MHz, $CDCl_3$) δ -127.3. LC-MS: t_r= 4.97 min, purity: 93.4%, M/z $[M+H]^+$ 388.

General procedure D:

(E)-4-(3-(Phenyldiazenyl)benzyl)-1H-imidazole (65)

To a stirred solution of protected imidazole **49** (1.05 g, 2.84 mmol) in EtOH (116 mL) was added 6M aq. HCl (14.2 mL, 85.3 mmol). The solution was heated for 16 h at 70 °C. After cooling to RT, 2.5M NaOH solution was added until pH 8 and the resulting mixture was extracted with EtOAc (3x30 mL). The combined organic phases were dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by flash column chromatography eluting with a gradient from 40:55:5 cyclohexane:EtOAc:TEA to 90:5:5 EtOAc:TEA:MeOH. The resulting solid was recrystallized from a H_2O:EtOH mixture. This yields the title compound as orange crystals (524 mg, 70%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 – 7.86 (m, 2H), 7.82 – 7.75 (m, 2H), 7.56 (s, 1H), 7.53 – 7.48 (m, 2H), 7.48 – 7.40 (m, 2H), 7.36 (d, *J* = 7.9 Hz, 1H), 6.79 (s, 1H), 4.06 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.0, 152.8, 140.9, 135.0, 131.6, 131.1, 129.4, 129.2, 123.1, 123.0, 121.3, 33.6. HMBC analysis indicates the presence of two additional carbon signals present at 117.2 and 136.9 ppm. LC-MS: t_r= 3.35 min, purity: >99%, M/z $[M+H]^+$ 263. HRMS calcd. for $C_{16}H_{15}N_4$ $[M+H]^+$ = 263.1297, found 263.1304.

(E)-4-(3-((2-Fluorophenyl)diazenyl)benzyl)-1H-imidazole (66)

This compound was synthesized according to general procedure **D**, using protected imidazole **50** (190 mg, 0.490 mmol), 6M aq. HCl (2.45 mL, 14.7 mmol) and EtOH (17.5 mL) as starting materials. This yielded the title compound as an orange solid (82 mg, 60%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 – 7.77 (m, 2H), 7.75 – 7.70 (m, 1H), 7.57 (s, 1H), 7.47 – 7.42 (m, 2H), 7.38 (d, *J* = 7.5 Hz, 1H), 7.29 – 7.23 (m, 1H), 7.21 (t, *J* = 7.7 Hz, 1H), 6.79 (s, 1H), 5.90 (bs, 1H), 4.06 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 160.2 (d, *J* = 257.6 Hz), 153.2, 141.0, 140.8 (d, *J* = 6.9 Hz), 135.0, 132.6 (d, *J* = 8.3 Hz), 132.1, 129.4, 124.5 (d, *J* = 3.8 Hz), 123.4, 121.6, 117.9, 117.2 (d, *J* = 19.9 Hz), 33.6. HMBC analysis indicates the presence of two additional carbon signals present at 116.9 and 136.9 ppm. ^{19}F NMR (235 MHz, $CDCl_3$) δ -124.5 LC-MS: t_r= 3.50 min, purity: >99%, M/z $[M+H]^+$ 281. HRMS calcd. for $C_{16}H_{14}N_4F$ $[M+H]^+$ = 281.1202, found 281.1209.

(E)-4-(3-((3-Fluorophenyl)diazenyl)benzyl)-1H-imidazole (67)

This compound was synthesized according to general procedure **D**, using protected imidazole **51** (725 mg, 1.87 mmol), 6M aq. HCl (9.36 mL, 56.1 mmol) and EtOH (66.8 mL) as starting materials. This yielded the title compound as an orange solid (358 mg, 68%).^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.60 – 7.53 (m, 2H), 7.50 – 7.45 (m, 1H), 7.44 (t, *J* = 7.7 Hz, 1H), 7.40 – 7.36 (m, 1H), 7.17 (tdd, *J* = 8.2, 2.7, 0.9 Hz, 1H), 6.79 (s, 1H), 4.06 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.4 (d, *J* = 247.7 Hz), 154.2 (d, *J* = 6.7 Hz), 152.7, 141.1, 135.1, 132.1, 130.4 (d, *J* = 8.5 Hz), 129.4, 123.3, 121.4, 120.6 (d, *J* = 2.8 Hz), 117.8 (d, *J* = 22.0 Hz), 108.1 (d, *J* = 22.8 Hz), 33.7. HMBC analysis indicates the presence of two additional carbon signals present at 116.6 and 137.0 ppm. ^{19}F NMR (235 MHz, $CDCl_3$) δ -112.1. LC-MS: t_r= 3.52 min, purity: 98.9%, M/z $[M+H]^+$ 281. HRMS calcd. for $C_{16}H_{14}N_4F$ $[M+H]^+$ = 281.1202, found 281.1210.

(E)-4-(3-((4-Fluorophenyl)diazenyl)benzyl)-1H-imidazole (68)

This compound was synthesized according to general procedure **D**, using protected imidazole **52** (262 mg, 0.676 mmol), 6M aq. HCl (3.38 mL, 20.3 mmol) and EtOH (24.2 mL) as starting materials. This yielded the title compound as a yellow solid (104 mg, 55%).^{1}H NMR (600 MHz, $CDCl_3$) δ 7.94 - 7.87 (m, 2H), 7.80 – 7.73 (m, 2H), 7.58 (s, 1H), 7.44 (t, *J* = 7.7 Hz, 1H), 7.37 (d, *J* = 8.0 Hz, 1H), 7.22 – 7.14 (m, 2H), 6.79 (s, 1H), 5.00 (bs, 1H), 4.06 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.5 (d, *J* = 252.0 Hz), 152.8, 149.3 (d, *J* = 3.0 Hz), 140.9, 135.0, 131.6, 129.4, 125.0 (d, *J* = 8.9 Hz), 123.1, 121.2, 116.2 (d, *J* = 22.8 Hz), 33.7. HMBC analysis indicates the presence of two additional carbon signals present at 116.9 and 137.0 ppm. ^{19}F NMR (235 MHz, $CDCl_3$) δ -109.4. LC-MS: t_r= 3.61 min, purity: >99%, M/z $[M+H]^+$ 281. HRMS calcd. for $C_{16}H_{14}N_4F$ [M+H]+ = 281.1202, found 281.1189**.**

(E)-4-(3-((2,6-Difluorophenyl)diazenyl)benzyl)-1H-imidazole (69)

This compound was synthesized according to general procedure **D**, using protected imidazole **53** (500 mg, 1.23 mmol), 6M aq. HCl (6.17 mL, 37.0 mmol) and EtOH (44.0 mL) as starting materials. This yielded the title compound as an orange solid (213 mg, 58%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 – 7.75 (m, 2H), 7.59 (s, 1H), 7.48 – 7.40 (m, 2H), 7.35 – 7.28 (m, 1H), 7.04 (t, *J* = 8.7 Hz, 2H), 6.80 (s, 1H), 4.61 (bs, 1H), 4.07 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.9 (dd, *J* = 258.9, 4.5 Hz), 153.6, 141.0, 132.6, 130.4 (t, *J* = 10.3 Hz), 129.5, 123.3, 121.3, 112.7 (dd, *J* = 20.0, 4.1 Hz), 33.6. HMBC analysis indicates the presence of three additional carbon signals present at 116.9, 134.5 and 137.3 ppm. ^{19}F NMR (235 MHz, $CDCl_3$) δ -121.8. LC-MS: t_r= 3.45 min, purity: >99%, M/z $[M+H]^+$ 299. HRMS calcd. for $C_{16}H_{13}N_4F_2$ $[M+H]^+$ = 299.1108, found 299.1099.

(E)-4-(3-((2-Chlorophenyl)diazenyl)benzyl)-1H-imidazole (70)

This compound was synthesized according to general procedure **D**, using protected imidazole **54** (398 mg, 0.985 mmol), 6M aq. HCl (4.93 mL, 29.6 mmol) and EtOH (24.6 mL) as starting materials. This yielded the title compound as a red solid (152 mg, 52%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 – 7.78 (m, 2H), 7.65 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.57 – 7.51 (m, 2H), 7.42 (t, *J* = 7.7 Hz, 1H), 7.40 – 7.34 (m, 2H), 7.34 – 7.29 (m, 1H), 6.77 (s, 1H), 4.05 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.1, 148.9, 141.1, 135.3, 135.1, 132.1, 131.8, 130.8, 129.4, 127.4, 123.9, 121.4, 117.7, 33.6. HMBC analysis indicates the presence of two additional carbon signals present at 117.1 and 137.0 ppm. LC-MS: t_r= 3.68 min, purity: 97.1%, M/z $[M+H]^+$ 297. HRMS calcd. for $C_{16}H_{14}N_4Cl$ $[M+H]^+$ = 297.0907, found 297.0906.

(E)-4-(3-((4-Chlorophenyl)diazenyl)benzyl)-1H-imidazole (71)

This compound was synthesized according to general procedure **D**, using protected imidazole **55** (572 mg, 1.42 mmol), 6M aq. HCl (7.08 mL, 42.5 mmol) and EtOH (35.4 mL) as starting materials. This yielded the title compound as a red solid (256 mg, 61%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8.6 Hz, 2H), 7.81 – 7.74 (m, 2H), 7.58 (s, 1H), 7.50 – 7.41 (m, 3H), 7.38 (d, *J* = 7.5 Hz, 1H), 6.79 (s, 1H), 4.63 (bs, 1H), 4.06 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 152.7, 151.0, 140.9, 136.9, 134.9, 131.7, 129.3, 129.3, 124.1, 123.1, 121.2, 33.5. HMBC analysis indicates the presence of two additional carbon signals present at 116.9 and 137.7 ppm. LC-MS: t_r= 3.90 min, purity: >99%, M/z $[M+H]^+$ 297. HRMS calcd. for $C_{16}H_{14}N_4Cl$ $[M+H]^+$ = 297.0907, found 297.0898.

(E)-4-(2-Methyl-3-(phenyldiazenyl)benzyl)-1H-imidazole (72)

This compound was synthesized according to general procedure **D,** using protected imidazole **56** (604 mg, 1.58 mmol), 6M aq. HCl (7.88 mL, 47.3 mmol) and EtOH (56.3 mL) as starting materials. This yielded the title compound as orange crystals (369 mg, 85%) ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.95 – 7.88 (m, 2H), 7.58 (s, 1H), 7.55 – 7.49 (m, 3H), 7.46 (t, *J* = 7.2 Hz, 1H), 7.33 – 7.28 (m, 1H), 7.22 (t, *J* = 7.7 Hz, 1H), 6.67 (s, 1H), 4.88 (bs, 1H), 4.08 (s, 2H), 2.66 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.1, 151.5, 139.5, 137.0, 134.8, 132.3, 130.9, 129.2, 126.3, 123.1, 114.2, 31.7, 13.2. HMBC analysis indicates the presence of two additional carbon signals present at 117.3 and 136.8 ppm. LC-MS: t_r= 3.71 min, purity: >99%, M/z $[M+H]^+$ 277. HRMS calcd. for $C_{17}H_{17}N_4$ $[M+H]^+$ = 277.1453, found 277.1457.

(E)-4-(2-Methyl-5-(phenyldiazenyl)benzyl)-1H-imidazole (73)

This compound was synthesized according to general procedure **D,** using protected imidazole **57** (320 mg, 0.834 mmol), 6M aq. HCl (4.17 mL, 25.0 mmol) and EtOH (30 mL) as starting materials. This yielded the title compound as orange crystals (167 mg, 72%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 (d, *J* = 7.2 Hz, 2H), 7.76 (d, *J* = 2.0 Hz, 1H), 7.72 (dd, *J* = 8.0, 2.1 Hz, 1H), 7.58 (s, 1H), 7.53 – 7.47 (m, 2H), 7.47 – 7.42 (m, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 6.69 (s, 1H), 4.04 (s, 2H), 2.36 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 152.9, 151.4, 140.4, 138.7, 134.8, 131.3, 130.9, 129.2, 124.2, 122.9, 121.2, 31.7, 19.7. HMBC analysis indicates the presence of two additional carbon signals present at 117.4 and 136.4 ppm. LC-MS: t_r= 3.63 min, purity: >99%, M/z $[M+H]^+$ 277. HRMS calcd. for $C_{17}H_{17}N_4$ $[M+H]^+$ = 277.1453, found 277.1454.

(E)-4-(3-Methyl-5-(phenyldiazenyl)benzyl)-1H-imidazole (74)

This compound was synthesized according to general procedure **D,** using protected imidazole **58** (604 mg, 1.58 mmol), 6M aq. HCl (7.88 mL, 47.3 mmol) and EtOH (56.3 mL) as starting materials. This yielded the title compound as orange crystals (212 mg, 49%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.90 – 7.85 (m, 2H), 7.60 (s, 1H), 7.57 (s, 1H), 7.55 – 7.47 (m, 3H), 7.47 – 7.42 (m, 1H), 7.16 (s, 1H), 6.78 (s, 1H), 4.00 (s, 2H), 2.39 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.0, 152.8, 140.8, 139.3, 135.0, 132.4, 131.0, 129.2, 122.9, 121.4, 120.8, 33.5, 21.4. HMBC analysis indicates the presence of two additional carbon signals present at 117.6 and 136.9 ppm LC-MS: t_r= 3.71 min, purity: >99%, M/z $[M+H]^+$ 277. HRMS calcd. for $C_{17}H_{17}N_4$ $[M+H]^+$ = 277.1453, found 277.1457.

(E)-4-(4-Methyl-3-(phenyldiazenyl)benzyl)-1H-imidazole (75)

This compound was synthesized according to general procedure **D,** using protected imidazole **59** (501 mg, 1.31 mmol), 6M aq. HCl (6.53 mL, 39.2 mmol) and EtOH (46.7 mL) as starting materials. This yielded the title compound as orange crystals (286 mg, 79%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.92 – 7.86 (m, 2H), 7.51 (s, 1H), 7.50 – 7.40 (m, 4H), 7.24 – 7.17 (m, 2H), 6.73 – 6.68 (m, 1H), 3.92 (s, 2H), 2.66 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.0, 150.7, 138.2, 136.6, 136.2, 135.0, 131.5, 131.4, 130.8, 129.1, 123.0, 117.5, 115.6, 33.1, 17.2. LC-MS: t_r= 3.70 min, purity: >99%, M/z $[M+H]^+$ 277. HRMS calcd. for $C_{17}H_{17}N_4$ $[M+H]^+$ = 277.1453, found 277.1460.

(E)-4-(3-(*O*-tolyldiazenyl)benzyl)-1H-imidazole (76)

This compound was synthesized according to general procedure **D,** using protected imidazole **60** (524 mg, 1.37 mmol), 6M aq. HCl (6.83 mL, 41.0 mmol) and EtOH (52 mL) as starting materials. This yielded the title compound as orange crystals (273 mg, 72%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.76 (d, *J* = 7.8 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.57 (s, 1H), 7.43 (t, *J* = 7.7 Hz, 1H), 7.38 – 7.30 (m, 3H), 7.27 – 7.22 (m, 1H), 6.79 (s, 1H), 5.85 (bs, 1H), 4.07 (s, 2H), 2.70 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.4, 150.9, 140.9, 138.2, 135.0, 131.4, 131.3, 131.0, 129.3, 126.6, 123.7, 120.9, 115.6, 33.6, 17.7. HMBC analysis indicates the presence of two additional carbon signals present at 117.2 and 137.1 ppm. LC-MS: t_r= 3.78 min, purity: >99%, M/z $[M+H]^+$ 277. HRMS calcd. for $C_{17}H_{17}N_4$ $[M+H]^+$ = 277.1453, found 277.1442.

(E)-4-(3-(*M*-tolyldiazenyl)benzyl)-1H-imidazole (77)

This compound was synthesized according to general procedure **D,** using protected imidazole **61** (620 mg, 1.62 mmol), 6M aq. HCl (8.08 mL, 48.5 mmol) and EtOH (66.8 mL) as starting materials.

This yielded the title compound as orange crystals (174 mg, 39%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79 – 7.72 (m, 2H), 7.72 – 7.67 (m, 2H), 7.51 (s, 1H), 7.43 – 7.35 (m, 2H), 7.33 (d, *J* = 7.4 Hz, 1H), 7.27 (d, *J* = 7.7 Hz, 1H), 6.76 (s, 1H), 4.03 (s, 2H), 2.44 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.0, 152.8, 141.0, 139.1, 136.8, 135.1, 131.9, 131.5, 129.3, 129.0, 123.1, 123.0, 121.1, 120.6, 117.5, 33.5, 21.5. LC-MS: t_r= 3.79 min, purity: >99%, M/z $[M+H]^+$ 277. HRMS calcd. for $C_{17}H_{17}N_4$ $[M+H]^+$ = 277.1453, found 277.1441.

(E)-4-(3-(*P*-tolyldiazenyl)benzyl)-1H-imidazole (78)

This compound was synthesized according to general procedure **D,** using protected imidazole **62** (620 mg, 1.62 mmol), 6M aq. HCl (8.08 mL, 48.5 mmol) and EtOH (66.8 mL) as starting materials. This yielded the title compound as orange crystals (358 mg, 80%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 – 7.70 (m, 4H), 7.55 (s, 1H), 7.42 (t, *J* = 7.4 Hz, 1H), 7.36 – 7.27 (m, 3H), 6.78 (s, 1H), 5.96 (bs, 1H), 4.05 (s, 2H), 2.43 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.2, 150.9, 141.7, 140.9, 135.0, 131.3, 129.9, 129.3, 123.0, 121.1, 33.6, 21.6. HMBC analysis indicates the presence of two additional carbon signals present at 117.4 and 136.9 ppm. HSQC analysis indicates the presence two overlapping carbons at 123.0 ppm. LC-MS: t_r= 3.78 min, purity: >99%, M/z $[M+H]^+$ 277. HRMS calcd. for $C_{17}H_{17}N_4$ $[M+H]^+$ = 277.1453, found 277.1437.

(E)-4-(2-Fluoro-5-(phenyldiazenyl)benzyl)-1H-imidazole (79)

This compound was synthesized according to general procedure **D**, using protected imidazole **63** (724 mg, 1.87 mmol), 6M aq. HCl (9.34 mL, 56.1 mmol) and EtOH (66.8 mL) as starting materials. This yielded the title compound as an orange solid (426 mg, 81%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.83 (bs, 1H), 7.89 – 7.80 (m, 3H), 7.80 – 7.74 (m, 1H), 7.54 (s, 1H), 7.50 – 7.41 (m, 3H), 7.14 (t, *J* = 8.9 Hz, 1H), 6.80 (s, 1H), 4.04 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.6 (d, *J* = 251.7 Hz), 152.6, 149.2 (d, *J* = 3.2 Hz), 136.0, 135.1, 131.1, 129.2, 128.0 (d, *J* = 17.2 Hz), 125.8 (d, *J* = 5.5 Hz), 122.9 (d, *J* = 9.0 Hz), 122.9, 116.7, 116.1 (d, *J* = 23.9 Hz), 27.0 (d, *J* = 2.0 Hz). ^{19}F NMR (471 MHz, $CDCl_3$) δ -114.0. LC-MS: t_r= 3.53 min, purity: 97.7%, M/z $[M+H]^+$ 281. HRMS calcd. for $C_{16}H_{14}N_4F$ $[M+H]^+$ = 281.1202, found 281.1199.

(E)-4-(4-Fluoro-3-(phenyldiazenyl)benzyl)-1H-imidazole (80)

This compound was synthesized according to general procedure **D**, using protected imidazole **64** (440 mg, 1.14 mmol), 6M aq. HCl (5.68 mL, 34.1 mmol) and EtOH (40.6 mL) as starting materials. This yielded the title compound as red crystals (273 mg, 86%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.92 (dd, *J* = 8.0, 1.3 Hz, 2H), 7.63 (dd, *J* = 7.1, 2.3 Hz, 1H), 7.58 (s, 1H), 7.53 – 7.44 (m, 3H), 7.37 – 7.30 (m, 1H), 7.18 (dd, *J* = 10.3, 8.5 Hz, 1H), 6.77 (s, 1H), 4.94 (bs, 1H), 3.99 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.0 (d, *J* = 256.6 Hz), 152.9, 140.5 (d, *J* = 7.0 Hz), 136.1 (d, *J* = 3.7 Hz), 135.0, 132.9 (d, *J* = 8.1 Hz), 131.6, 129.3, 123.3, 117.8, 117.2 (d, *J* = 20.1 Hz), 33.2. HMBC analysis indicates the presence of two additional carbon signals present at 116.4 and 137.6 ppm. ^{19}F NMR (471 MHz, $CDCl_3$) δ -127.8. LC-MS: t_r= 3.55 min, purity: >99%, M/z $[M+H]^+$ 281, HRMS calcd. for $C_{16}H_{14}N_4F$ $[M+H]^+$ = 281.1202, found 281.1193.

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